# Acta Obstetricia et Gynecologica Scandinavica

Published by

The Scandinavian Association of Obstetricians and Gynæcologists

Editor

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Sabbåtsbergs kvinnoklinik 113 82 Stockholm Sweden

Vol 54, 1975

Printed in Sweden by
Almqvist & Wiksell Uppsala 1975

# INDEX

Letters Å and A—see under A letters Ø and Ö—see under O

rum alkaline phosphatase reflects nonspecifically the rate of bone growth (22)

Adrenal androgens are present in small amounts during childhood. The urinary 17 ketosteroids found prior to puberty are primarily derived from cortisol degradation At puberty there is a marked increase in urinary 17 ketosteroids These are derived from the adrenal androgens (15 24) The early pubescent changes are influenced by the adrenal androgens and are non specific for the male and female affecting the early development of axillary and pubic hair acne long bone growth and protein anabolism

Puberty is expressly the time in a child s development when appraisal of the development should be based on a determination of the biological age The determination can be made either by anthropometric methods e g on the basis of the development of pubic hair or chemically e g on the basis of the excretion of steroids In complicated cases the entire hormonal status should often be determined and norms are required for this assessment Information on the changes in the hormonal status of healthy girls before and after the menarche and their correlations with the development of sex characters is still incomplete on many points The purpose of this part of the present study was to investigate the behaviour before and after the menarche of certain hormones affecting the linear growth the growth hormone 17 ketosteroids 17 hydroxy corticosteroids and alkaline phosphatase

## SERIES AND METHODS

The study was made as a cross-sectional investigation of 146 schoolgirls and nurses The clinical examination of all the subjects was carried out by one of the authors (R L K) All the girls were healthy as judged from their medical histories and clinical examinations Heights and weigh s ranged from 122 3 to 175 0 cm and from ?1 3 to 7? 0 kg respectively When the means were plotted onto the Finnish standard curve (1) it was seen that both the height and weight means fell very close to the 50th percentile curve

A hand X ray was taken of all the girls for the determination of bone age This was done using the Tanner–Whitehouse–Healy method (?6) which has been found eminently suitable for Finnish children (28 9) All determinations were carried out by one of the authors (R L K) who was familiar with the method The skeletal ages of the series ranged from 8 0 to adult (chronological ages from 7 0 to 20 years)

The stage of puberty was determined by c inl al examination according to breast and publ hair ratings as described by Tanner (2?) In the girls whose menarche had taken place less than 6 months previous the mean for the breast stage was 3 44 (S D 0 5) and the mean pubic hair stage 3 39 (S D 0 6) The mean age of menarche of all the menstruating girls was 1 .2 (S D 1 6) years The skeletal age of the girls who had menstruated for less than 6 months was 1. 8 years and the chronological age 1. 9 years

The purpose of the study was to group the girls, as precisely as possible according to their biological maturity For this reason the bone age of the premenarcheal girls was used since it correlates better with the menarche than the chronological age (13 14) The grouping of the menstruating girls was based on the so-called gynecological age which refers to the time that had passed from the menarche (32) In the later presentation of the results the date of the menarche is indicated in a break in the curves On the basis of the foregoing the date of menarche seems occur at approximately the 13th year of bone age and therefore the relevant curves presumably can be combined

The 24 hour urine was collected by the girls at home according to meticulous instructions 17 KS and 17 OHCS were determined at the Helsinki University Institute of Medical Chemistry using the Peterson & Lettrer method (17) for the former and the method described by Few for the latter (4)

The alkaline phosphatase was determined at the laboratory of the Children s hospital of the Helsinki University Central Hospital using the Sommer method (?1)

A blood specimen for the determination of the growth hormone was taken in the afternoon between 15 and 16 o clock Since the series consisted mostly of school girls this ensured a uniform state of moderate fast The children had had a meal at school at about 11 o clock and fasted thereafter The blood was centrifuged immediately it had been drawn and the serum was deep-frozen It was not thawed until the moment of determination in Uppsala Growth hormone in serum was assayed by a radioimmunosorbent technique (30 31) using human pituitary growth hormone ( 0) and rabbit anti human pituitary growth hormone The results were expressed in ng per ml The mean GH level in serum of 20 healthy women aged from 17 to 36 years was 8 ng/ml with $\pm$ S E M 2 36–3 3 ng/m

In order to determine the mean levels and variations of hormone serum level or urinary excretion the subjects were grouped according to ske etal age gyne o ogical age or stage of puberty as described by Tanner (? ) The distributions of GH 1? KS and 17 OHCS in all groups were found to be skewed and had to be norma ized by logarithmic transformation Means standard de ia tions and standard errors of the mean were ca u a ed from the transformed values For a ka ne phosp atase values no transformation was needed and arithmeti m a s were calcu ated

## RESULTS

### Growth hormone

This investigation comprised 100 girls. All d t r minations gave values above zero and th rang

*Table I Growth hormone according to skeletal age* n°/ml

| Skeletal age y | N | Mean | M– SEM | M+ SEM |
|---|---|---|---|---|
| 8–9 9 | 9 | 3 69 | 3 01 | 4 53 |
| 10–10 9 | 9 | 4 27 | 3 56 | 5 13 |
| 11–11 9 | 20 | 6 62 | 5 54 | 7 92 |
| 12–12 9 | 30 | 5 46 | 4 75 | 6 27 |
| 13–13 9 | 11 | 7 70 | 5 83 | 10 10 |
| 14–14 9 | 14 | 4 68 | 3 59 | 6 08 |
| 15 adult | 7 | 2 70 | 1 99 | 3 66 |

for the whole series was 0 7–23 5 ng/ml. Table I gives the means ±S E M in groups based on bone age It is seen that the deviations are wide especially in the groups aged 11–14 years The highest mean value 7 70 ng/ml was obtained for the girls of a bone age of 13 years All these 11 girls had begun to menstruate and the peak height velocity was passed The lowest growth hormone levels were obtained for the girls with bone age under 10 years and for those of over 15 years The combined mean value for those aged 8–10 years and those aged over 14 years differed significantly from that of the age group 11–13 years ($t = 2\ 80$ $P < 0\ 01$) The finding suggests a transient activation of growth hormone production around the menarche

Fig 1 shows the results of the growth hormone determinations timed against the date of menarche Age groups premenarche were still based on bone age but postmenarche were based on the gynecological age It can be seen that the curve has a maximum around the time of the menarche the rise beginning from the start of the 11th year of age The mean values were lowest for the youngest non menstruating girls and for those with the longest history of menstruation The mean for all the premenarcheal girls was 5 45 ng/ml and that for the postmenarcheal girls 5 18 ng/ml the difference of the means is not statistically significant.

The separate curves have been drawn in Fig 2 according to the breast rating and the pubic hair rating The peaks coincide with pubic hair rating 2 and breast rating 3 This corresponds relatively well with the time just before the menarche

*Alkaline phosphatase*

The alkaline phosphatase of 145 girls in the series was determined from blood samples Table II

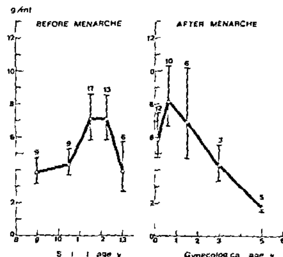



*Fig 1* Growth hormone levels in pre and postmenarcheal girls

presents the arithmetic mean values and standard errors of means by groups based on bone age The mean value for the girls of an average bone age of 11 5 years is seen to be the highest 5 85 B L units while the mean value is lowest in the adult bone age group 1 93 B L units The highest alkaline phosphatase activity therefore coincides with the time about one year before the mean menarcheal age of this series

Fig 3 shows the changes in alkaline phosphatase around the menarche A distinct rise is seen in premenarche beginning at about 10 5 years and lasting for a year A declining trend begins immediately before the menarche and is

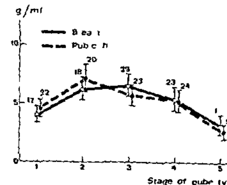



*Fig 2* Growth hormone levels according to the stages of puberty

Table II *Alkaline phosphatase according to skeletal age B–L units*

| Skeletal age y | N | Mean | S D | S E M |
|---|---|---|---|---|
| 8–9 9 | 8 | 4 59 | 1 07 | 0 38 |
| 10–10 9 | 9 | 4 61 | 1 89 | 0 63 |
| 11–11 9 | 19 | 5 85 | 1 47 | 0 34 |
| 12–12 9 | 26 | 5 47 | 1 92 | 0 38 |
| 13–13 9 | 40 | 4 92 | 1 84 | 0 27 |
| 14–14 9 | 19 | 3 03 | 1 25 | 0 28 |
| 15–15 9 | 16 | 2 45 | 1 00 | 0 25 |
| 16–16 9 | 1 | 2 70 | — | — |
| Adult | 7 | 1 93 | 0 56 | 0 21 |

already very close to the adult level only 1 5 years after the menarche

In Fig 4 curves have been drawn according to the breast and the pubic hair ratings The highest means coincide with ratings 1 and 2

*17 ketosteroids*

The 24-hour excretion of 17 ketosteroids was studied in 146 girls Table III presents the results by groups based on bone age It is found that in the youngest age groups the excretion is at a level of 1 mg/24 hours and rises as bone age advances to adulthood to a level of 4 mg/4 hours The deviations are moderate There to be no sudden acceleration in the increase of excretion The level is higher among

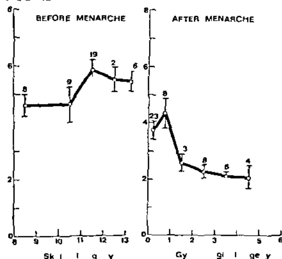



*Fig 3* Alkaline phosphatase in pre and postmenarcheal girls

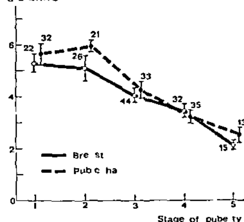



*Fig 4* Alkaline phosphatase according to stages of puberty

those of adult bone age than in the preceding age group

Fig 5 shows the mean values of pre and post menarcheal girls The premenarcheal mean value was 1 79 mg/24 h and the postmenarcheal 3 31 mg/24 h The difference is statistically highly significant ($p<0\,001$) The mean values for the girls with a bone age of 12–13 years at a level of 2 5 mg/24 h are almost identical with those of the girls who had menstruated for 0–1 years The mean value for those who had menstruated for more than 5 years was 5 60 mg/24 h which is higher than the mean for the girls of adult bone age (Table III) This means that the excretion of 17 ketosteroids seems to increase even after the bone age has reached the adult level according to the present study more than 5 years after the menarche

Fig 6 presents the change in the excretion of 17 ketosteroids by groups based on breast and

Table III *Urinary excretion of 17 ketosteroids according to skeletal age mg/24 h*

| Skeletal age y | N | Mean | M− S E M | M+ S E M |
|---|---|---|---|---|
| 8–9 9 | 11 | 0 98 | 0 80 | 1 18 |
| 10–10 9 | 18 | 1 43 | 1 29 | 1 58 |
| 11–11 9 | 25 | 1 74 | 1 58 | 1 91 |
| 12–12 9 | 36 | 2 69 | 2 46 | 2 94 |
| 13–13 9 | 21 | 3 50 | 3 14 | 3 90 |
| 14–14 9 | 21 | 3 18 | 2.85 | 3 55 |
| 15–adult | 14 | 4 04 | 3 41 | 4 76 |

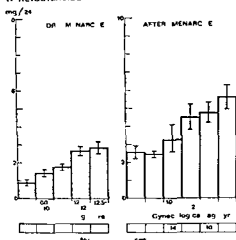



*Fig 5* 17 ketosteroid excretion in pre and postmen archeal girls

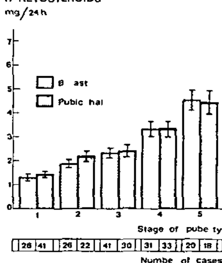



*Fig 6* 17 ketosteroid excretion according to stages of puberty

pubic hair ratings the mean values increase al most linearly as secondary sex characters develop The girls whose breasts had not yet developed have a mean 17 KS excretion of 1 18 mg/24 h When the development of the breasts had reached stage 5 the excretion was 4 50 mg/24 h i e a four fold increase The means are practically the same also on the basis of pubic hair rating

### *17 hydroxycorticosteroids*

17 hydroxycorticosteroids were determined from the same 146 urines as the 17 ketosteroids The mean values and standard errors of the means classified according to the bone age are presented in Table IV It is seen that the increase is less than that for the 17 KS The mean value for the youngest group 3 29 mg/24 h is approx imately doubled as the bone age reaches the adult level

Fig 7 presents the hormone excretion in pre and postmenarche The mean value for all the premenarcheal girls was 4 59 mg/24 h and for the menstruating girls 6 63 mg/24 h the difference was not great but it was statistically highly sig nificant ($p<0\ 001$) The adult level was reached immediately after the menarche

If the situation is reviewed on the basis of breast and pubic hair ratings (Fig 8) the find ing is somewhat contradictory as the breast rat ing increases the mean value of the 17 OHCS excretion seems to increase from a level of 4 mg/24 h to 7 10 mg/24 h almost linearly For pubic hair rating on the other hand the steep-est increase is from rating 1 to rating 2 and the mean value for the fully developed girls exceeds those for the above ratings

Table V shows the coefficients of correlation for the quantities studied calculated on the total series to the bone age stage of puberty height and weight It can be seen that alkaline phos-phatase correlations mostly are significantly nega tive and the 17 KS and 17 OHCS correlations significantly positive whereas the GH correla tions for the total series do not significantly differ from zero

## DISCUSSION

The part played by growth hormone in the human growth spurt during puberty is still obscure Heald

Table IV *Urinary excretion of 17 OHCS according to skeletal age mg/24 h*

| Skeletal age y | N | Mean | M− S E M | M+ S E M |
|---|---|---|---|---|
| 8–9 9 | 11 | 3 29 | 2 87 | 3 87 |
| 10–10 9 | 18 | 3 91 | 3 58 | 4 27 |
| 11–11 9 | 25 | 4 50 | 4 06 | 4 99 |
| 1 -12 9 | 36 | 6 34 | 5 86 | 6 86 |
| 13–13 9 | 21 | 6 60 | 6 14 | 7 09 |
| 14–14 9 | 21 | 6 49 | 5 99 | 7 04 |
| 15–adult | 14 | 5 87 | 5 30 | 6 53 |

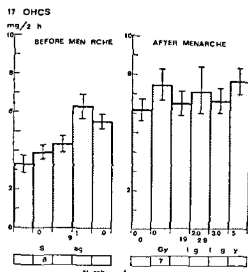



*Fig 7* 17 hydroxycorticosteroid excretion in pre and postmenarcheal girls

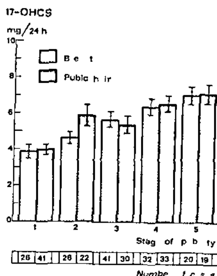



*Fig 8* 17 hydroxycorticosteroid excretion according to stages of puberty

claims that growth hormone is not required for the adolescent growth spurt (9) The argument in favour of this assertion is that dwarfs who have no growth hormone at all show a delayed but regular spurt Tanner does not find the mat ter clarified the cause of the ending of long bone growth can scarcely be explained by the action of a single hormone it marks the end of a long process Probably many factors are in volved (23) Other steroids most likely andro gens are in all probability needed in addition to the growth hormone (7 10)

The growth hormone level varies in all individ uals within wide limits from hour to hour and from one time of day to another (8 9 11) The conditions in which samples are taken must there fore be carefully standardized before the limits

e V *Correlation coefficients for the total series (No 146)*

| | Bone age | Height | Weight | Breast stage | Pubic hair stag | Alkaline phosph | 17 keto-steroids | 17 OHCS | Growth hormone | Total estro gens |
|---|---|---|---|---|---|---|---|---|---|---|
| Bone age | — | | | | | | | | | |
| Height | 838*** | — | | | | | | | | |
| Weight | 743*** | 830 | — | | | | | | | |
| Breast stage | 825** | 780 | 750* * | — | | | | | | |
| Pubic hair stage | 804** | 778 | 755*** | 860 * | — | | | | | |
| Alkaline phosphatase | – 443 | – 345** | – 372 | – 402* | – 436 | — | | | | |
| 17 keto steroids | 499 ** | 469 | 517* | 521 * | 508 | – 301 * | — | | | |
| 17 OHCS | 351 | 388 | 419 | 397* | 38 | – 120 | 738 * | — | | |
| Growth hormone | 128 | 132 | 027 | 100 | 064 | – 043 | 057 | 019 | — | |
| Total estrogens | 453 | 357 ** | 325 * | 430 * | 421 | – 344 | 407 | 81 | 110 | — |

*P*<0 05 *P*<0 01 * *P*<0 001

of normal variations can be drawn In the present study the conditions were not perfectly controlled e g as regards the duration and degree of fasting and for this reason the results obtained can only be considered tentative Since samples were taken from all age groups in identical conditions the mean hormone levels in the different stages of puberty can be compared The mean value curves showed a significant rise at the 11th year of bone age and at the same time the mean height curve of the series also indicated that an accelerated height growth began about this time This might support the hypothesis according to which the growth spurt is a combined action of GH and androgens The mean GH serum level of 17–36 year old women (2 8 ng/ml) was significantly ($p<0 01$) lower than the mean for girls during puberty and approximately equals the values for girls who have menstruated for 4 years. This is another point to suggest that growth hormone production is accelerated at the age of puberty

The means of alkaline phosphatase seemed to be high already before the secondary sex characters had developed They began to fall before stage 3 was reached They coincided with the height spurt in its early phase as described earlier (3 27)

Reports in the literature of studies of the 17 ketosteroid excretion in adolescent girls are incomplete and do not cover the whole period of puberty Prout & Snaith found that the mean 24 hour excretion for girls aged 11–17 years was about 5 mg (18) They did not study the relationship between excretion and the stage of puberty Pennington & Dewhurst examined premenarcheal girls and showed that the mean 24-hour excretion of girls of an average age of 14 was of the magnitude of 3 mg and increased with advancing age (16) Tanner says that "the published data are scanty particularly about the time of adolescence and presents a partly estimated mean value curve in which the mean excretion of 10–15 year old girls increases from 2 to 6 mg/24 h (23) The results of the present study agree with all these earlier results but show that the adult level of 17 ketosteroid excretion is not reached until more than 5 years after the menarche that is to say even later than the adult level of estrogens Around the menarche one year before and one year after the mean values are practically the same This may indicate that a steady period in hormone excretion takes place about the time of the menarche

The 24-hour excretion of 17 OHCS in adults has been studied e g by Reddy according to whom the mean excretion was 4 7 mg/24 h with a range of 1 1–10 7 mg/24 h (19) Gardner & Snaith said that the excretion of girls aged 2–17 years was 3 1–2 0 mg/24 h (6) Pennington & Dewhurst in their series of premenarcheal girls found that the excretion increased with age and averaged 6 mg/24 h for girls of 13 (16) In the small series of Kowarski et al the excretion in girls aged from 9 to 17 years ranged from 1 7 to 7 0 mg/24 h (12) The present results concur well with those reported

The urinary secretion of cortisol metabolites is found to correlate well with surface area (2 12 24) In the present series it could be shown that the mean weight of the girls of the post menarcheal group hardly increased any more with age and the height increase also was slight The mean value curve for 17 OHCS closely follows the shape of the mean value curve for weight and height In premenarche a distinct increase is noted in the 13th year of age and the adult level is reached immediately after the menarche

Summarizing according to the present study no drastic changes in the production of growth hormone and adrenocortical hormones take place in the premenarcheal period but there is a summation of minor changes in the production of the various hormones

## ACKNOWLEDGEMENT

We are indebted to Sisko Asp Mag.Phil for the statistical treatment of the material

The study was supported by grants from Sigrid Juselius Foundation and the Ministry of Education in Finland and the Swedish Medical Research Council (grant No 16x-637)

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3 Clark, L Jr & Beck, E Plasma alkaline

Table I

| Series | Dose (ml) | Test dose (ml) | Depth (mm) | Site (o clock) 4 | 5 | 7 | 8 | *Cervix* | *Intra amniotic* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 5+2 | 2 | 2 | + | | | | | + |
| 2 | 5 | | 2 | | + | | | | |
| 3 | 5 | | 2 | | | + | | | |
| 4 | 5 | | 2 | | | | + | | |
| 5 | 5 | | 2 | | | | + | | |
| 6 | 5+5 | | 2 | | | | | ++ | |
| 7 | 2+4+4 | [illegible] | 2 | + | | +/+ | | | |
| 8 | 5+5+5 | | 2/6 | | + | | +/+ | | |
| 9 | 5 | | 4 | | | | | + | |
| 10 | 5+5 | | 4/8 | | | + | | + | |
| 11 | 5 | | 4 | + | | | | | |
| 12 | 5 | | 6 | | + | | | | |
| 13 | 5 | | 6 | | +[b] | | | | |
| 14 | 5 | | 6 | | | | | + | |
| 15 | 5 | | 6 | | | | | + | |

Medial direction
[b] Tangential direction

(16) Excellent results based on large series have also been reported (6 8)

In the main it is agreed that for PCB local anaesthesia should be administered in a low dose (25% of the maximum) and concentration It should be distributed at four injection sites in order to avoid the risk of a too large single intra ¹ r dose in the lower uterine segment or in fetal head The injection technique has always described as simple Emphasis has been laid n placing the injection a sufficient distance from the fetal head and from the lateral pelvic wall the cannula should therefore be introduced high in the lateral fornix tangentially to the fetal head So far no systematic study has been made of the spread of the anaesthetic agent in the para cervical tissue and the absorption from there The present work is an attempt of visualize with radio logical contrast the spread of the anaesthetic agent when deposited at different depths and in different sites in the paracervical space

## MATERIAL AND METHOD

For the radiological study 20 women who had been admitted to the clinic for legal abortion were chosen *The period of pregnancy varied from 14 to 19 weeks* (mean 16) The age of the patients varied from 34 to 45 years (mean 37) They had stated that further pregnancies were not contemplated in the immediate future In all cases the pregnancies were terminated by the extra amniotic instillation of 0 1% Rivanol® solution via Foley catheter which was left *in situ* The catheter cuff was filled with 20 ml physiological NaCl solution The Rivanol dose was adjusted according to the duration of the pregnancy at 10 ml/pregnancy week

Bupivacaine 0 5% with the addition of adrenaline 1 200 000 (Marcaine®–Adrenaline) 0 5% was used as local anaesthetic To make it possible to visualize the injection of the anaesthetic into the tissues equal parts *of the X ray contrast medium Isopac Cerebral® 780 mg* I/ml was used as diluting fluid instead of ordinary physiological NaCl solution 5 ml of this solution (=bu pivacaine 0 25% adrenaline 1 400 000 Isopac Cerebral 700 mg I) was used as a standard dose at each in jection site

The anesthetic was deposited with a Kobak cannula at different sites and at different depths as described in Table I In principle only one injection was given per patient partly to avoid confluence and additive effects of anaesthetic deposited a other sites and partly *to allow the radiological follow up of the spread and* absorption of the anaesthetic to be carried out for as long as possible The position of the cannula was checked by TV fluoroscopy before the injection All the films were taken in an antero-posterior *direction in supine* position with Philips 70 mm technique In individual cases a lateral picture was taken as well

The radiographs were taken with maximum velocity of 4 radiographs/sec The radiation dose to the ovaries was approximately 10 millirads in one exposure The maximum number of exposures per patient was 20 with a total maximum gonad dose of 0 2 rad/patient (4 15)

## RESULTS

The anaesthetic was deposited in eight patients 2 mm deep in a total of ten sites in one case the dose was repeated one hour later In all cases the an

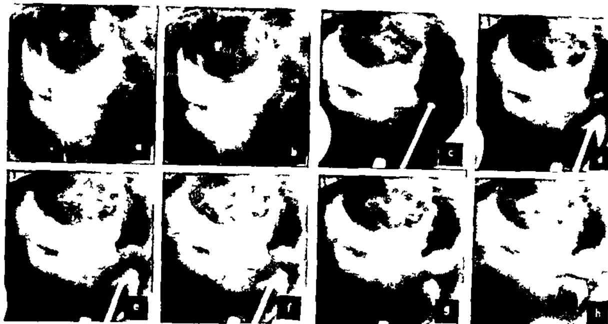
aesthetic was retained for at least one hour No spread to surrounding venous plexus could be demonstrated at any stage during the radiological follow up Throughout the study the injected doses were absorbed slowly and completely In Series 1 (Fig 1) the contrast was injected intra amniotically (Figs 1*a–b*) to mark the size of the uterine cavity and the proximity to the paracervical test dose (2 ml) at 4 o clock (Fig 1*c*) One hour later remaining contrast is still found at the two sites (Fig 1*h*) In no instance was reflux noted from the site of the injection after the removal of the Kobak cannula The doses which were at first sharply delimited (Figs 1*c–e*) gradually usually after a few minutes became blurred in contour and were dispersed only slowly during the next hour (Fig 1*h*) The close-up in Series 5 (Fig 5*e*) illustrates this where the sharp contour of the injected fluid is seen without the slightest sign of infiltration due to early venous absorption No difference in absorption between 4 5 7 and 8 o clock could be established Series 1–5 7–8 (Figs 1–5 7–8) A repeated dose after the primary test dose resulted in a similar spread of the deposited anaesthetic There was no reflux through the earlier injection site (Series 7 Fig 7) The same series also shows how the anaesthetic is deposited in a concentric semicircle bilaterally (Fig 7*k–l*) On injection into the cervix (Series 6 Fig 6*b–c*) the anaesthetic spread around between the fascia and the vaginal epithelium After a second dose the same tissue spaces were refilled with swelling of cervical os A little parametrial spread could be discerned but there was no absorption into the venous plexus (Fig 6*d–f*)

The anaesthetic was deposited at a depth of 4 mm in the following sites in three patients (*a*) Cervix (Series 9 Fig 9) Because a little parametrial spread had taken place from a depth of 2 mm without positive signs of venous absorption the same amount (5 ml) was injected to a depth of 4 mm Some venous absorption is seen immediately during the injection (Figs 9*a–b*) but it did not increase despite the dose being doubled (Fig 9*e*) (*b*) In Series 10 (Fig 10) the injection depth in the cervix was increased to 8 mm at the same time as a further deposit was made at a depth of 4 mm at 7 o clock Under great resistance a track was first formed in the cervix (Figs 10*a–b*) Suddenly the resistance ceased and the entire dose was shortly afterwards seen deposited in the Pouch of Douglas (Figs 10*d–e*) A lateral picture taken immediately afterwards verified the finding (Fig 10*f*) Penetration of the peritoneal reflection had taken place The dose deposited at 7 o clock showed here a little spread to the venous plexus (Fig 10*f*) (*c*) In Series 11 (Fig 11) the anaesthetic was deposited at a depth of 4 mm at 4 o clock (Figs 11 *a–b*) but the needle entered the cervix medially instead of tangentially Here a concentric deposit round the cervix with obvious if not marked venous absorption was seen immediately

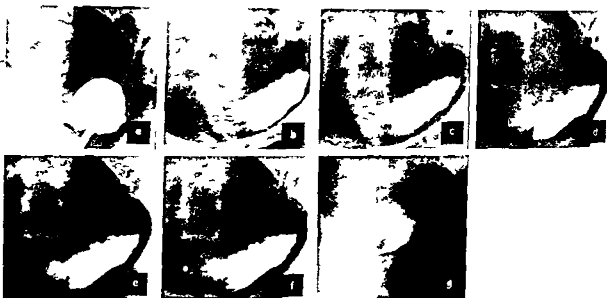
The anaesthetic was deposited at a depth of 6 mm in five patients (*a*) In Series 8 (Fig 8) an injection was first made at 8 and 5 o clock at a depth of 2 mm (Figs 8 *a–b*) Six minutes later an equally large dose (5 ml) was again deposited at 8 o clock but at a depth of 6 mm (Fig 8 *d*) This latter dose spread to a larger area and dispersed considerably more rapidly The patient also had a feeling of numbness in the right leg as is to be expected because of the lateral injection site On the lateral picture (Fig 8 *e*) the point of the needle was seen quite separate from the first injection (*b*) In Series 12 (Fig 12) the anaesthetic was injected at a depth of 6 mm at 5 o clock at a slight medial angle (Figs 12 *a–c*) Massive spread to the venous plexus was noted here and also contralaterally (Figs 12 *j–l*) Four exposures per second in a total of 3 seconds (*c*) In Series 13 (Fig 13) the anaesthetic was deposited at the same site and at the same depth as in Series 12 but the needle here ran tangentially Despite this rapid and massive spread was seen to the veins of the same side (Figs 13 *b–d*) and to those of the contralateral side (Figs 13 *e–i*) Thirty minutes later contrast was still retained (Fig 13 *j*) Obviously the surplus during the first 5 min had diffused into the vessels because of high extravascular pressure (*d*) and (*e*) In Series 14 and 15 (Figs 14 and 15) the injections were directed into the cervix In Series 14 somewhat more laterally whereby some venous spread could be detected In Series 15 only parametrial spread was seen without corresponding venous spread

## DISCUSSION AND CONCLUSION

The present radiological study has shown clearly that the injection depth is of the greatest im

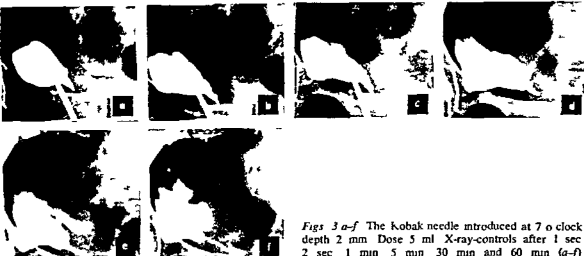


*Figs 1 a–h* Marking of the intra amniotic space with test dose 5 ml (*a–b*) The Kobak needle is introduced at 4 o clock depth 2 mm (*c*) Dose 2 ml X ray-controls after 1 2 4 20 and 60 min (*d–h*)



*Figs 2 a–g* The Kobak-needle introduced at 5 o clock depth 2 mm Dose 5 ml X-ray-controls after 5 10 15 30 and 60 min (*b–f*) Lateral picture (*g*)

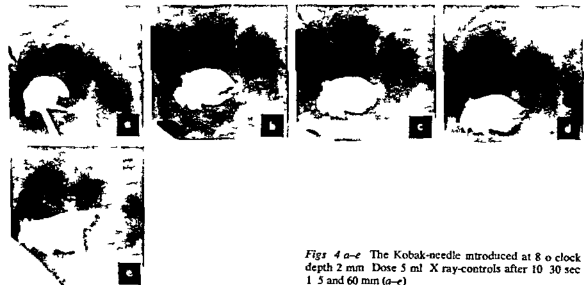
At a depth of 4 mm at each injection site venous absorption can already be established although it is not so marked and complete as at greater injection depths (Series 9 Figs 9 *a–b* Series 10 Figs 10 *i–k* Series 11 Figs 11 *f–l*) A depth of 2 mm (max 3 mm) which corresponds to the thickness of the vaginal epithelium did not produce visible venous spread in any patient (Series 1–7 and 8 Figs 1–7 and 8 *a–c*) The explanation for this could be that the anaesthetic was depostited immediately into the space that lies between epithelium and the paracervical



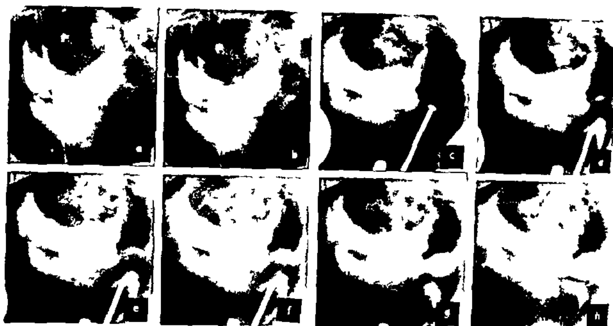
*Figs 3 a–f* The Kobak needle introduced at 7 o clock depth 2 mm Dose 5 ml X-ray-controls after 1 sec 2 sec 1 min 5 min 30 min and 60 min (*a–f*)

vessels From there the anaesthetic diffused out into the loose intervascular connective tissue instead of—as happens at greater injection depths—being deposited directly within the paracervical venous plexus where the risk of intravascular injection is extremely great The patients in the experimental series were in the 14th to 19th week of pregnancy when the anatomy is not the same as at the end of the third trimester The formation of the lower uterine segment which alters the vaginal fornix and the cervix the increasing vascularity and elasticity of the paracervical tissues and the effect of labour on the circulation are the most essential differences On the other hand the thickness of the vaginal epithelium shows no great difference This factor has been seen to be of fundamental importance and justifies the choice of patients At a depth of 2–3 mm apart from the risk of intervascular injection there is practically no risk of injection into the cervical substance the lower uterine segment and the fetal head Similarly the risk of depositing the anaesthetic close to the nervous plexuses of the pelvic wall is also extremely slight

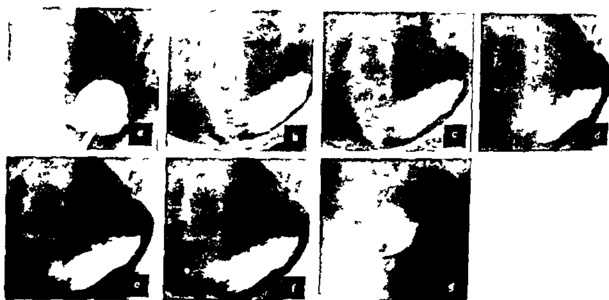
Particularly with deep elastic fornices the tissues between the vaginal wall and the pelvic wall can easily be compressed so that the distance is reduced to a few millimetres Here too the subepithelial injection technique eliminates the risk of faulty deposition of the anaesthetic preventing in



*Figs 4 a–e* The Kobak-needle introduced at 8 o clock depth 2 mm Dose 5 ml X ray-controls after 10 30 sec 1 5 and 60 min (*a–e*)

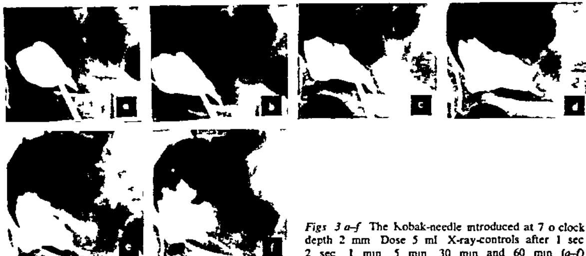


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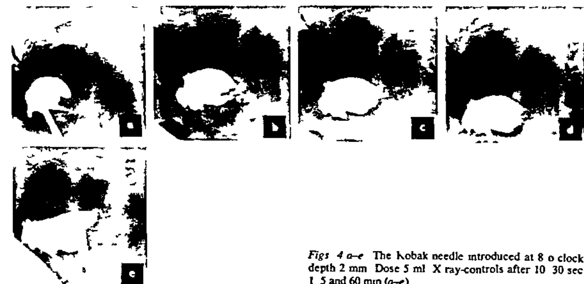
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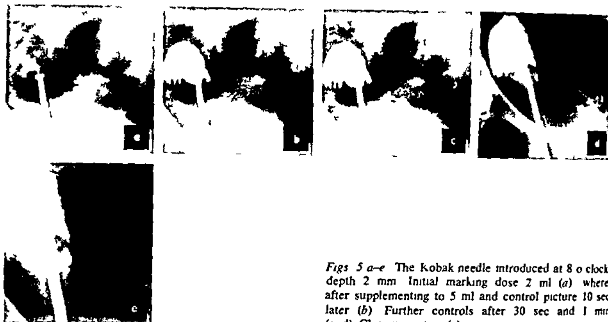

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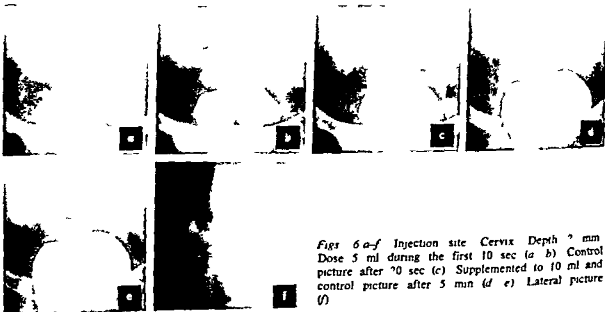

*Figs 4 a–e* The Kobak needle introduced at 8 o clock depth 2 mm Dose 5 ml X ray-controls after 10 30 sec 1 5 and 60 min (*a–e*)



*Figs 5 a–e* The Kobak needle introduced at 8 o clock depth 2 mm Initial marking dose 2 ml (*a*) where after supplementing to 5 ml and control picture 10 sec later (*b*) Further controls after 30 sec and 1 min (*c d*) Close-up picture (*e*)

jections which have no effect or which result in paraesthesiae

In Series 11 increased venous spread with the needle angled medially was seen even at an in jection depth of 4 mm (Figs 11 *f–l*) This experience should show the value of keeping the injecting needle strictly tangential to the presenting l part even when an injection depth of 2 mm is at In many cases especially early in labour the lateral fornices can be asymmetrical because of an eccentric cervix In these cases it can be difficult to deposit the anaesthetic symmetrically in the proper site Symmetry is easily established by digital correction of the position of the cervix Flowers (2 3) has also injected into the cervix with good effect However the author has not mentioned the frequency and importance of possible effects on the fetus If here too a depth of 2 mm is maintained the risk to the fetus should be minimal (Series 6 Fig 6) Some venous absorption is found even at a depth of 4 mm (Series 9 Figs 9 *a–e*) but to a considerably lesser extent than when in jections are made at other sites at the same depth Injection into the cervical substance during the



*Figs 6 a–f* Injection site Cervix Depth ? mm Dose 5 ml during the first 10 sec (*a b*) Control picture after ?0 sec (*c*) Supplemented to 10 ml and control picture after 5 min (*d e*) Lateral picture (*f*)




*Figs 7 a–l* The Kobak needle introduced at 7 o clock depth 2 mm Dose 2 ml Four pictures/sec during the first second (*a–d*) Thereafter two pictures/sec during the next second (*e f*) The needle is then introduced at 4 o clock depth 2 mm Dose 4 ml with a picture rate of 2/sec during the first second (*g h*) Control picture 30 sec and 1 min later (*i j*) Repeat of primary dose at 7 o clock with 4 ml at 2 mm depth thereafter control 5 and 20 min later (*k l*)

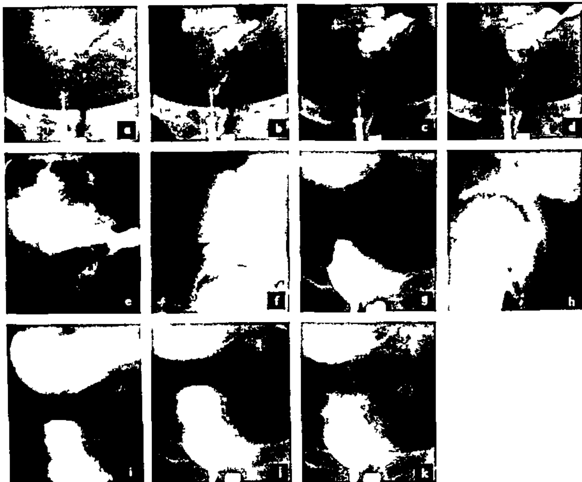
progress of labour when the presenting fetal part lies in the immediate vicinity seems less attractive because the needle if only a slightly angled can perforate the cervical substance with the risk of transferring the anaesthetic directly into the fetus Flowers injection technique thus does not offer any advantage over injection in the lateral fornix In those cases where venous spread of the anaesthetic could be seen it took place only during the first few seconds after the injection (Series 9 10 11 12 13 14) Thereafter the anaesthetic gradually thinned out similar to what happens with slow capillary absorption It has been demonstrated by Steffensen et al (20) that diffusion can occur both through the walls of the veins and through the walls of the arteries The speed of the absorption is considerably greater at an injection depth of 6 mm than at 2 mm (Series 8) Large doses of anaesthetic (≥50% of the maximum dose) injected at only two sites and without regard to the depth of injection must lead to a considerable risk of massive venous transfer at the beginning of the injection particularly if the anaesthetic is deposited very quickly This can obviously explain the rather discouraging results reported by some authors (16 21 22)

Parametriography has formerly been done on *non pregnant* patients by Ingelman Sundberg (10 11) The injection depth varied from 20 to 30 mm without any signs of spread to the venous plexus So far no parametriography on *pregnant* patients has been performed and the present work shows

*Figs 8 a–k* Primary depositing of 5 ml at 5 o clock at 2 mm depth Thereafter a further 5 ml deposited 8 o clock at 2 mm depth Immediately afterwards ures/sec were taken (*a b*) Controls 5 and later (*c d*) Lateral picture (*e*) where the injection depth was increased to 6 mm 5 ml was injected at this depth whereafter 2 pictures/sec were immediately taken (*f g*) Controls after 1 5 15 and 30 min (*h–k*)



*Figs 9 a–e* Injection site Cervix Depth 4 mm Dose 5 ml 1 picture/sec during the first two seconds (*a b*) Lateral picture (*c*) Controls after 10 min (*d*) Supplementing of dose to 10 ml and control 1 min later (*e*)



*Figs 10 a–k* Injection site Cervix Depth 8 mm Dose 5 ml ₄ pictures/sec for 2 sec (*a–d*) Spreading 1 min after initial dose (*e*) Lateral picture immediately after (*f*) At 7 o clock deposited 5 ml at 4 mm depth antero-posterior and lateral picture (*g h*) Control pictures 1 2 and 5 min after the injection (*i–k*)

that this difference in injection depth between non pregnant and pregnant patients is of particular importance

The best injection technique is as follows The patient lies supine with her knees and hip joints well flexed and her hips fully abducted The operator places himself to the right of the patient with his left shoulder towards the head of the bed and he palpates the cervix with his left hand In this way

Table II *Composition of the series*

| Part | No of patients | No of blocks |
|---|---|---|
| II | 44 | 68 |
| III | 90 | 136 |
| Total | 134 | 204 |

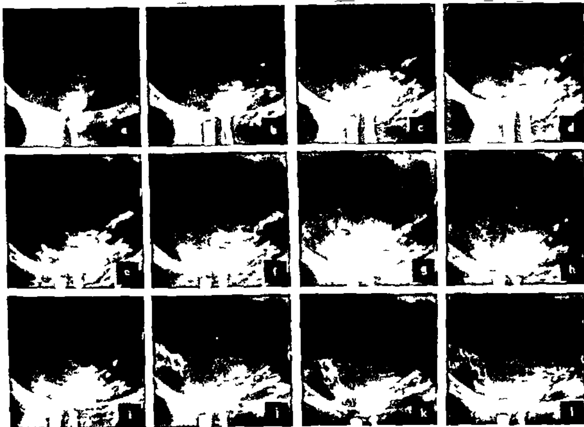
the Kobak needle can be introduced without the movement being cramped and without the operator running the risk of having his arms and hands crossed Two fingers are inserted between the presenting part of the fetus and the cervical wall thus avoiding injection into the fetus (12) Aspiration with the syringe before the injection must be regarded as an obligatory safety measure

My preliminary views of the importance of the injection depth were presented by Dr G Hedberg from this clinic at the international symposium in Bad Aachen in June 1970 for which purpose two pictures from Series 13 were loaned (5) The final results were not ready for presentation until the meeting of the East Swedish Gynecological Association on 4th September 1971

*Figs 8 a–k* Primary depositing of 5 ml at 5 o clock at 2 mm depth Thereafter a further 5 ml deposited 8 o clock at 2 mm depth Immediately afterwards were taken (*a b*) Controls 5 and later (*c d*) Lateral picture (*e*) where the injection depth was increased to 6 mm 5 ml was injected at this depth whereafter 2 pictures/sec were immediately taken (*f g*) Controls after 1 5 15 and 30 min (*h–k*)



*Figs 9 a–e* Injection site: Cervix Depth 4 mm Dose 5 ml 1 picture/sec during the first two seconds (*a b*) Lateral picture (*c*) Controls after 10 min (*d*) Supplementing of dose to 10 ml and control 1 min later (*e*)




*Figs 12 a–l* The Kobak needle introduced at 5 o clock at slight medial angle Injection depth 6 mm Dose 5 ml 4 pictures/sec for 3 sec (*a–e*) 2 pictures/sec for 6 sec after beginning of injection (*f–g*) Control and pictures 7 10 15 20 and 30 sec later (*h–l*)

each After the last block and depending on the length of labour two groups were obtained These are dealt with in Part II and Part III respectively

One group presented in Part II consists exclusively of patients where delivery took place within one hour of the last PCB It was possible here to make a simultaneous determination of acid base status and Apgar score while the PCB was still effective

The appraisal of Apgar score by the team of midwives was confirmed biochemically (1) which led to a more uniform estimate of Apgar score in the other cases presented in Part III (Deliveries later than 1 hour after the last PCB ) A control series consisting of uncomplicated deliveries in which neither analgesics nor sedatives were used has similarly been appraised by Apgar score and determination of acid base status

## PART II

### MATERIAL AND METHOD

The series consists of 44 patients with normal cardio vascular and respiratory status Patients with late toxaemia diabetes or signs of *fetal distress before* analgesia were excluded from the series The total number of PCB in the series was 68 The last anaesthetic was injected in all cases immediately before the cervix was fully dilated irrespective of whether the patient was still free from pain because of the preceding injection—with the object of extending the duration of the anaesthetic beyond the moment of birth Blood tests for acid base status were taken as soon as the umbilical cord was visible and before the child had time to scream The first sample was taken from the artery by means of a *heparinized syringe* Immediately afterwards the umbilical vein was punctured similarly At the same time an assisting midwife took sample from the mother using a heparinized cannula previously introduced into the cubital vein The midwife awarded points to the infants after 1 and 10 min according to Apgar without knowledge of acid-base status The series was com

Table VII *Acid base status in mother and child in control group and experimental group*
Apgar score 9 and 10 Average value ±S D

| | | | Mother | | | Child Venous | | | Child Artery | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | No | Age | pH | $CO_2$ | −BE | pH | $CO_2$ | −BE | pH | $CO_2$ | −BE | Duration (h) | Weight (g) | Length (cm) |
| Control | 10 | 27 1 ±5 0 | 7 37 ±0 06 | 33 9 ±4 0 | 4 3 ±4 2 | 7 37 ±0 04 | 34 8 ±6 4 | 4 2 ±3 5 | 7 34 ±0 03 | 40 6 ±5 7 | 3 7 ±2 6 | 5 7 ±2 7 | 3 804 ±619 | 51 ±? |
| Experimental | 37 | 26 9 ±4 8 | 7 36 ±0 08 | 30 9 ±7 1 | 7 5 ±3 5 | 7 35 ±0 06 | 34 5 ±6 9 | 6 0 ±2 6 | 7 32 ±0 07 | 38 4 ±7 ?3 | 6 3 ±3 2 | 6 4 ±4 9 | 3 581 ±455 | 51 ±? |
| *p* | | NS | NS | NS | <0 05 | NS | NS | NS | NS | NS | <0 05 | NS | NS | NS |

technique are identical with those in Part II The team of midwives was the same It must therefore be presumed that the obstetrical management of the patients at delivery was the same as in Part II Similarly the assessment of Apgar score was identical Consideration has here been given to the effect and duration of the paracervical injections and their number per patient variations in the fetal heartsounds the presence of meconium in the amniotic fluid obstetrical complications and operative methods The total number of anaes thetics in the series was 136

## RESULTS

### *algesic effect*

The effect and average duration of the blocks and their number per patient is shown in *Table VIII* The result is almost analogous with that presented in Part II In 93 4% of the total of 136 PCB in jections the effect was Good or Excellent There was no significant difference between the durations of repeated PCB The more or less unsuccessful injections numbered 6 6% and showed a clear concentration in certain patients (Table IX)

### *Effect on the fetus*

The distribution of Apgar score at 1 min and 10 min postpartum is seen in Table X According to Apgar score asphyxia was discovered in 6 cases (Apgar score ≤7)

### *Case description*

*Apgar score 7/10* 23 year-old primigravida PCB × 1 with duration 130 min Delivery 155 min after PCB had ceased Breech presentation Fetal heart rate 130–150 beats/min

*Apgar score 7/9* 2? year-old primigravida PCB × 1 with duration 70 min Delivery 68 min after PCB had ceased Rather long second stage (48 min) Fetal heart sound frequency 1?0–150 beats/min Cephalic presentation

*Apgar score 6/8* 26 year-old primigravida PCB × 1 with duration 70 min Delivery 129 min after PCB had ceased Breech presentation

*Apgar score 3/9* 25 year-old para ? PCB × 1 with duration 140 min Delivery 30 min after PCB had ceased Breech presentation

*Apgar score 3/8* 35 year-old para 2 PCB × ? with duration 140 min+60 min Delivery 30 min after the effect of the last anaesthetic had ceased Occipito-posterior cephalic presentation+shoulder dystocia Hy drammos Fetal weight 4810 g

*Apgar score 2/8* ?0 year-old primigravida PCB × 2 with duration 120 min+75 min 60 min after PCB had ceased onset of bradycardia to 80 beats/min be tween the last two expulsive contractions Normal amniotic fluid

No case of bradycardia in relation to PCB

Table VIII

| | No of blocks | | | | | Total no of blocks |
|---|---|---|---|---|---|---|
| Order of the blocks | 1 | 2 | 3 | 4 | 5 | |
| Effect | | | | | | |
| Excellent | 80 | 34 | 3 | 1 | 1 | 119 |
| Good | 6 | 1 | 1 | | | 8 |
| Poor | 4 | 3 | 2 | | | 9 |
| Total blocks according to order | 90 | 38 | 6 | 1 | 1 | 136 |
| No of re peated blocks | 73 | 34 | 4 | | | |
| Average duration (min) S D | 105 31 | 100 37 | 1?6 36 | 110 - | | |

Table IX

| No of patients | No judged poor anaesthesia per patient |
|---|---|
| 4 | 1 |
| 1 | 2 |
| 1 | 3 |

Bradycardia (frequency≤1?0 beats/min during delivery occurred in 5 cases in the group with normal Apgar score (10–8)

*Case descriptions*

*Apgar score 8/9*  6 year-old primigravida  PCB × 2 with duration 1?5 min+210 min  180 min after PCB had ceased  onset of bradycardia to 80 beats/min 15 min later  delivery with VE

*Apgar score 8/10*  20-year-old primigravida  PCB × 3 with duration 60 min+135 min+100 min  Bradycardia 100 min after the last PCB had been injected  10 min later delivery with VE

*Apgar score 8/10*  23 year-old primigravida  PCB × 3 with duration 1?5 min+45 min+110 min  Delivery 30 min after the last anaesthetic had ceased  Bradycardia during the last 10 min of the second stage

*Apgar score 9/9*  ?4 year-old primigravida  PCB × ? with duration 70 min+170 min  Bradycardia to 50 beats/min for 15 min 50 min after the first PCB  Bradycardia only in dorsal position  disappeared immediately in lateral position  Possible vena cava syndrome

*Apgar score 9/10*  23 year-old gravida ?  PCB × 1 with duration 95 min  Fetal heart rate normal 60 min after PCB had been injected  the amniotic fluid became meconium stained  Normal delivery 40 min later

No case of tachycardia was discovered

Vacuum extractions  VE was done in 13 cases

| Number | Indication |
|---|---|
| 9 | Secondary uterine inertia |
| 2 | Bradycardia during second stage (Case descriptions No 1 and 2) |
| 2 | Protracted second stage *Humanitarian indications* |

Caesarean section ? cases  Indication  feto pelvic disproportion

## GENERAL CLINICAL DISCUSSION

*Analgesic effect*

Of a total of 204 blocks  the analgesic effect was Good or Excellent in 192 cases (94 1%) which is at least 10% better than with previous investigators (8 17 22 25) although there must be some reservation because of the subjectivity of such assessments  In addition  consideration must be given to the fact that in the present work  the dose was only a total of 25 mg of anaesthetic agent and was thus 50% lower than that usually employed  The average duration was also comparable  Neither was there any significant difference between the duration of anaesthesia and the need for repeated injections

Table X *Apgar score*

| No of patients | Apgar score at 1 | Apgar score at 10 |
|---|---|---|
| 8 | 10 | 10 |
| 48 | 9 | 10 |
| 12 | 9 | 9 |
| 9 | 8 | 10 |
| 4 | 8 | 9 |
| 3 | 8 | 8 |
| 1 | 7 | 10 |
| 1 | 7 | 9 |
| 1 | 6 | 8 |
| 1 | 3 | 9 |
| 1 | 3 | 8 |
| 1 | 2 | 8 |
| 90 | | |

*Side effects in the mother*

None of the mothers showed any symptoms whatsoever  Numbness in the legs  haematoma  parametritis  cramps and collapse  and so on  have been described (8 9 16)  These can be regarded as results of intravascular injections  high dosage  but mainly as *lack of knowledge of the importance of the injection depth*  Uterine inertia occurred only during the first 10 min after PCB and was not interpreted as a disadvantage  Zourlas & Kumar have reported weak uterine contractions for 30–40 min in 50% of the cases (26)

*Effect on the fetus*

In Part II  all the children were born under the influence of PCB  If there were signs of fetal asphyxia during the course of the delivery  a relation could be found with PCB  In the group with Apgar score 8–10  there was an instance of transitory bradycardia and one of tachycardia  both mild  Both Apgar score and acid base status were good  The group with Apgar

score 7 included only 2 cases both with good agreement with acid-base status As seen from the case description asphyxia suddenly appeared in one case at the end of the second stage caused by a tight umbilical cord which was divided at the vulva In the second case there was the onset of a slowly progressive asphyxia 30 min after PCB Here too the case history suggests that a relation with PCB is improbable

In Part III all cases with Apgar score ≤7 developed signs of fetal asphyxia at the earliest 30 min after the PCB effect had ceased In the group with normal Apgar score (8–10) bradycardia occurred in 4 cases and meconium-stained amniotic fluid in 1 case Only in one of these did the bradycardia begin while the PCB was having its effect Bradycardia occurred only in the dorsal position and could be made to disappear in the lateral position As this relation with position could be provoked a few times there was obviously a vena cava syndrome Where meconium appeared in the amniotic fluid it was not until 60 min after PCB had been given Fetal bradycardia has also been observed at PCB with placebo (17 24) Bradycardia caused by PCB according to Jung et al (13) has its onset 2–23 min after the injection and according to Rogers (18) 2–10 min after The criterion for bradycardia, according to Rogers (18) is a cardiac heart frequency of ≤120 beats/min for more than 30 sec If we follow these recommendations PCB did not cause asphyxia in any instance in the series Vasicka et al (23) studied 41 patients with catheters for PCB lying in the parametrium In 5 cases bradycardia occurred in connection with the injection. X-ray-contrast was injected via the catheters and in 4 patients it was established that the point of the catheter lay in the myometrium The contrast was here seen to disappear rapidly into the circulation whereas it was retained on the contralateral side where the catheter lay correctly At caesarean section PCB was given via catheters partly laid into the myometrium in 6 patients In 5 of these a similar radiological finding was obtained This too suggests that PCB-induced bradycardia appears to be due to the injection and that the injection depth is of the greatest importance As seen from the results presented here no serious complications have arisen that could be ascribed to the paracervical block The correlation between Apgar score and acid-base status in the umbilical cord is good and confirms that the assessment of the vitality of the infants included in the series was correct

## ACKNOWLEDGEMENT

I wish to thank AB Bofors Nobel Pharma, Molndal Sweden for support in this investigation

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*Submitted for publication February 8 1973*

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Acta Obstet Gynec Scand 54 29–32 1975

# THE EFFECT ON FERTILITY OF LIGATION OF THE LEFT SPERMATIC VEIN IN MEN WITHOUT CLINICAL SIGNS OF VARICOCELE

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*Abstract* Improvement of sperm qualities and fertility after operation for varicocele is well-documented The effect does not appear to relate to the degree of dilata tion of the spermatic veins For this reason 22 men with severely impaired fertility but without any clinical sign of varicocele were offered operation i e ligation of the left spermatic vein(s) Fertility was evaluated by re peated sperm examinations On average the childless ness lasted for 3 5 years before the operation took place A sperm examination was performed on average 6 3 months postoperatively In half the cases improvement of sperm quality was found In 9 cases no change was observed and in one man the sperm quality was re duced In 7 cases conception occurred within 16 months The improvement of both sperm quality and the con ception rate were highly significant in comparison with a non-operated control group with the same severely impaired fertility and without clinical sign of varicocele

In 1934 Wilhelm (18) and in 1944 Hammen (10) reported on a possible relation between varicocele and male infertility especially due to oligospermia and asthenospermia Since then many authors have demonstrated an improvement in sperm quality af ter ligation of the spermatic veins (3 4 8 9 12 13 15 17 18) Thus it seems probable that a rela tion exists between varicocele and male infertility but the mechanism involved is not known for cer tain Many theories have been put forward Se veral authors claim that the temperature of the scrotal contents is decreased after the operation causing improved spermiogenesis (4 6 17) but others (16 18) could find no change in the scrotal temperature after the surgical procedure Some (3 12) have postulated a postoperatively decreased venous pressure causing an increased oxygen ten sion A third possibility is a venous reflux of blood from the adrenal through the left spermatic vein caused by the anatomic conditions where this vein enters the left renal vein Metabolic products in the adrenal blood are supposed to impede spermio genesis The presence of such a reflux and ana stomoses between the vessels of the left and the right testis have been claimed in several papers (8 12 and others) However increased amounts of adrenal steroids have not been found in blood from the spermatic vein even in cases of varicocele (1 5 11)

Cases of both large and slight varicocele can respond well to ligation of the spermatic vein for example Dubin & Amelar (7) found no relation be tween the degree of varicocele and the improve ment in sperm quality after operation of 111 cases the sperm was improved in 81% and pregnancy ensued in 48% Palti et al (14) tried to improve the sperm by ligation of the spermatic vein in cases where no varicocele existed An improve ment of only short duration was observed and no pregnancy occurred Reviewing this material it ap pears to us to include cases where no improvement could be expected (cases of aspermia etc )

As the sperm even in cases of slight varicocele can be improved by operation and as practically no other therapeutic measures exist it was con sidered worthwhile to carry out a series of ligation of the left spermatic vein in men who had sperm with severely decreased fertility but no varicocele

Table I

| Decrease in fertility | Sperm concentration (mill /ml) | Motility | Immobile sperms | Abnormal sperm heads (%) |
|---|---|---|---|---|
| I None | 60 | ++++ | <40 | <25 |
| II Slight at least one quality is reduced as follows | 15–60 | ++++ | 40–80 | 25–35 |
| III Moderate at least one quality is reduced as follows | 5–15 | ++++ | 40–80 | 35–50 |
| IV Severe at least one quality is reduced as follows | <5 | +++–++–+ | 80–90 | 50–100 |
| V Very severe all sperm qualities are reduced | <5 | ++–+–O | ≤100 | 65–100 |
| Total | 0 | | | |

Table II *Pregnancy rate*

| | Group with surgery | Control group |
|---|---|---|
| Pregnancy | 7 (32 %) | 2 (5 %) |
| No pregnancy | 15 (68 %) | 42 (95 %) |

## MATERIAL

The series consists of 22 men where at least two semen specimens had shown decreased fertility All specimens were investigated in the same laboratory and for the classification the criteria shown in Table I were used Twenty-one patients were classified as cases of severely or very severely reduced fertility and one as moderately reduced All the female partners were also investigated and no abnormalities of major importance were found The infertility of the couples had existed for 3.5 years on average (1–6 years) New sperm specimens

Table III *Spermiograms before and after operation*

| Case no | Percentage of immobile sperms | | Motility | | Sperm concentration (mill/ml) | | Percentage of abnormal sperms | |
|---|---|---|---|---|---|---|---|---|
| | Before op | After op | Before op | After op | Before op | After op | Before op | After op |
| 1 | 36 | 34 | ++ | +++ | 14 | 76 | 61 | 43 |
| 2 | 36 | 33 | ++ | ++ | 56 | 74 | 53 | 38 |
| 3[a] | 46 | 35 | ++ | ++++ | 39 | 78 | 64 | 75 |
| 4 | 48 | 74 | + | + | 1 0 | 1 5 | 68 | 78 |
| 5 | 66 | 48 | ++ | ++ | 53 | 41 | 79 | 89 |
| 6 | 35 | 40 | +++ | ++++ | 189 | 160 | 59 | 75 |
| 7 | 74 | 67 | + | ++ | 6 6 | 29 | 77 | 7 |
| 8 | 31 | 34 | +++ | ++++ | 51 | 77 | 33 | 37 |
| 9 | 32 | 24 | +++ | ++++ | 79 | 38 | 46 | 4 |
| 10 | 65 | 33 | + | + | 40 | 59 | 3 | 35 |
| 11 | 38 | 34 | +++ | ++++ | 81 | 101 | 78 | 65 |
| 12[a] | 39 | 60 | ++ | ++ | 15 | 6 | 66 | 6 |
| 13 | 57 | 48 | ++ | ++ | 9 1 | 4 8 | 63 | 40 |
| 14 | 59 | 46 | ++ | ++ | 17 | 8 6 | 56 | 5 |
| 15 | 61 | 38 | +++ | +++ | 33 | 9 | 49 | 57 |
| 16[a] | 24 | 30 | ++++ | ++++ | 72 | 75 | 69 | 31 |
| 17 | 76 | 56 | ++ | ++ | 79 | 44 | 44 | 41 |
| 18 | 44 | 42 | + | ++++ | 0 6 | 38 | 68 | 35 |
| 19[a] | 59 | 37 | ++ | ++++ | 37 | 1 9 | 47 | 43 |
| 20 | 75 | 74 | + | ++ | 1 5 | 0 8 | 86 | 6 |
| 21 | 37 | 36 | ++ | ++++ | 40 | 74 | 86 | 6 |
| 22 | 27 | 38 | +++ | ++++ | 16 | 46 | 62 | 63 |

Cases where conception occured

were investigated on average 6 3 months (3–14 months) after the operation The couples have been observed for an average of 16 months (7–40 months) since the su gical procedure

As controls a group of 44 similar couples was inves tigated from the period immediately before the intro duction of surgery The variation in sperm qualities in specimens taken on average each 7 7 months (3–14 months) was noted and the number of pregnancies was investigated

*Surgical procedure*

All operations were carried out by one of us (P F A) Through an inguinal approach the cord was identified at the superficial inguinal ring and isolated for at least 2 cm towards the abdomen where the spermatic vein(s) was ligated and divided In patients with dilated crema steric veins or significant anastomoses between the two venous systems additional ligation of these vessels was done at the l vel of the pubic tubercle

All operations went normally Biopsies of both testes were obtained in most cases

## RESULTS

Pregnancy ensued in 7 (32%) of the couples from 5 to 14 months after the operation (average 9 5 months) whereas only 2 (5%) pregnancies oc curred in the control group within the same pe riod of observation (Table II) Using the $\chi^2$

| Testicular biopsy Spermatogenesis | | Comparison of sperm qualities before and after operation |
|---|---|---|
| Right | Left | |
| Not performed | | Improved |
| Normal | Normal | Improved |
| Normal | Normal | Improved |
| Decreased | Decreased | Unchanged |
| Normal | Normal | Unchanged |
| Normal | Normal | Unchanged |
| Decreased | Decreased | Improved |
| Normal | Decreased | Improved |
| Not performed | | Improved |
| Normal | Normal | Unchanged |
| Normal | Normal | Improved |
| Normal | Normal | Unchanged |
| Not performed | Decreased | Unchanged |
| Normal | Normal | Unchanged |
| Normal | Normal | Unchanged |
| Normal | Normal | Improved |
| Normal | Normal | Decreased |
| Not performed | | Improved |
| Normal | Normal | Improved |
| Decreased | Decreased | Unchanged |
| Not performed | Decreased | Improved |
| Decreased | Not performed | Improved |

test the difference is highly significant (0 01>$p$ >0 001)

A comparison of the sperm qualities before and after the operation is shown in Table III

In the 7 cases where pregnancy ensued hhe moti lity was improved in 4 men and unchanged in 2 in the seventh case the motility was ++++ already before operation Among the 15 couples where no pregnancy occurred the motility was improved in 8 men and unchanged in 7 The concentration of sperm in the pregnancy group was increased in 4 cases unchanged in one and decreased in 2 cases In the group without pregnancies the figures were 7 increased 3 unchanged and 5 decreased Con cerning the percentage of immobile sperms in the pregnancy group a decrease was found after the operation in 3 cases no changes in 2 and an in crease in 2 cases

For the non pregnancy group the corresponding figures were decreased 7 cases unchanged 4 and increased 4 cases In the pregnancy group the percentage of abnormal sperms after the operation was found decreased in 3 cases unchanged in 2 and increased in 2 cases The figures for the non pregnancy group were decreased 5 cases un changed 7 and increased 3 cases In conclu sion it must be said that improvement of no single quality could be related to pregnancy However the general classification was improved in the preg nancy group in 5 cases and unchanged in 2 whereas the same figures for the non pregnancy group were increased 7 cases unchanged 7 and decreased 1 case Testicular biopsy was per formed on 19 patients The results are shown in Table III In 7 cases spermatogenesis was de creased and in those no pregnancies ensued The evaluation was quantitative and not repeated post operatively so for that reason definite conclusions are difficult It should be noticed that in one case (no 7) the spermiogram was remarkably improved postoperatively although severely reduced sperma togenesis was found in both testes

Table IV shows the effect of the operation on the spermiogram and for comparison the spontane ous development is illustrated by the spermiograms of the control group taken with an interval of approximately the same magnitude as the interval between the pre and postoperative spermiograms in the former group Using the $\chi^2$ test the differ ence between the two groups is highly significant (0 01>$p$>0 001)

Table IV *Change in classification of spermiograms in the group with surgery and the control group*
Figures in parentheses are percentages

| Classification | Group with surgery | Control group |
|---|---|---|
| *Improved* | *12 (55)* | *8 (18)* |
| Unchanged | 9 (41) | 30 (68) |
| Decreased | 1 (4) | 6 (14) |

## DISCUSSION

Therapeutic measures to improve semen of men with decreased fertility are few and those available will only be helpful in a few cases Ligation of the spermatic vein is the only procedure that benefits in a significant number of cases Until now this operation has only been carried out in patients with varicocele but our results seem to indicate that even without incompetent veins the semen may be improved However so many unknown factors may be of importance that for definite conclusions to be drawn a much larger series will be needed

If our results are confirmed it may indicate that the ligation of the vein that matters rather the restoration of a better circulation Why ne ligation is of benefit is obscure but it could be the presence of a toxic substance in the left spermatic vein blood If so this substance may have its origin in the adrenal but the results of steroid analyses contradict this theory

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*Submitted for publication July 11 1973*

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Acta Obstet Gynec Scand 54 33–39 1975

# PHOSPHOLIPIDS IN AMNIOTIC FLUID

## II *Lecithin Fatty Acid Patterns related to Gestation Maternal Disease and Fetal Outcome*

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*Abstract* Amniotic fluid samples were extracted with chloroform and methanol for lipids Thin layer chromatography (TLC) was used to separate the phospholipids Lecithin spots from the TLC were scraped and methylated for fatty acid (FA) analysis by gas liquid chromatography (GLC) The FA composition of the lecithins from 61 samples of amniotic fluid were determined These included 17 from diabetic mothers 36 from Rh sensitized mothers and 8 from normal or pre-eclamptic mothers at gestational ages from 6–4 weeks The percentage of saturated FA increased from 58–70% at gestational ages of 26–31 weeks to 75–90% after 37 weeks gestation Palmitic acid comprised 24–51% myristic acid 0–4% stearic acid 18–44% and oleic acid 16–32% of the lecithin FA between 6–31 weeks gestation With increasing gestational age the percentage of palmitic and myristic acids increased while the percentage of stearic and oleic acid decreased Five of 27 infants delivered within seven days of obtaining the amniotic fluid samples and between 4–4? weeks gestation developed RDS The lecithins from the five patients with RDS had no myristic acid and decreased palmitic acid (4 of 5<60%) compared with healthy infants of similar gestational age The amniotic fluid lecithin from the five infants with RDS had 16–35% stearic acid while only two of 22 healthy infants had >9% stearic acid (11 & 17%) The ratio of stearic acid to oleic acid (SA/OA) was >1 3 in all five infants with RDS and <1 3 in all healthy infants Amniotic fluid from infants born between 34 and 38 weeks gestation who develop RDS has not only decreased Five of 27 infants delivered within seven days characteristic FA patterns associated with decreased surfactant activity The percentage of stearic acid more closely parallels pulmonary maturity than the concentration of lecithin or the L/S ratio

Low concentrations of lecithin in the amniotic fluid of infants who develop the respiratory distress syndrome (RDS) have been reported by several investigators (2 8 11 19 22) The lecithins from amniotic fluid when present in concentrations over 1 5 to 2 0 μM/100 ml contain largely saturated fatty acids primarily palmitic acid (1 8) Little data is available on the fatty acid composition of the lecithins found in low concentration in amniotic fluid between 26 and 35 weeks gestation Since all infants with a low concentration of lecithin in their amniotic fluid do not develop RDS there may be qualitative differences in the lecithins which would identify the infant who will develop RDS from the infant who will have normal lung expansion Such differences because of their effect on surface tension characteristics might identify the infant more likely to develop respiratory distress syndrome To answer this question the present study of the fatty acid composition of lecithins in amniotic fluid from infants with and without RDS was undertaken

## METHODS AND MATERIALS

Amniotic fluid was obtained by amniocentesis or by collection at the time of delivery Fluid contaminated with blood or meconium was discarded The concentrations of phospholipids in the samples were reported previously (14) Of the 61 samples analyzed 17 were from diabetic patients 36 from Rh sensitized patients and 8 were from patients with other obstetric problems There were 17 samples from 14 diabetic patients Thirteen of these 17 samples were obtained within 7 days of delivery Of the 22 patients with Rh sensitization 6 delivered within 7 days of obtaining the sample Seven of the eight samples from mothers with other complications of pregnancy were taken at delivery of within 7 days of delivery

All organic solvents were Analytical Reagent Grade Hexane used to extract methyl esters of fatty acids was Purum grade >99 mol% obtained from Fluka AG The transmethylating reagent used was 14% Boron Trifluoride Methanol purchased from Applied Science Laboratories Thin layer chromatography plates were spread using Merk silica gel H (45 g/100 ml water) at the wet thickness of 500 $\mu m$ The plates were air dried for 8–12 hours then oven dried at 110°C for 12 hours and desiccated over Drierite until used Thin layer chromatographic lipid standards were purchased from Sigma Chemical Company

*Extraction of phospholipids*

As in the previous report (14) the proportions of Bligh & Dyer (3) were followed accurately to insure maximum recovery of phospholipids with a minimum of water soluble contaminants remaining in the chloroform extract These proportions are methanol/chloroform/water M/C/W (2 1 0 9) which gives a single phase for extraction A final proportion of M/C/W (2 2 1 8) yields a phase separation with minimal water in the chloroform phase To 4 ml of amniotic fluid 10 ml methanol were added shaken and centrifuged at 2500 rpm for 5 minutes The supernatant was decanted into a separatory funnel and the precipitate was washed two times with 5 ml chloroform decanting the chloroform into the methanol extract and shaking each time With the first chloroform wash added to the methanol and shaken initial proportions are achieved M/C/W (2 1 0 9)

After the second chloroform wash and with the addition of 4 5 ml water the final proportions are obtained M/C/W (2 2 1.8) The lower layer (chloroform extract) was drawn off and evaporated to dryness on a rotary evaporator The sample was quantitatively transferred with methanol and chloroform to a small sample tube made from the barrel of a pasteur pipette and evaporated to dryness under $N_2$ The sample was redissolved in a small quantity of chloroform (25λ) to be spotted on a TLC plate

*Thin layer chromatography (20)*

TLC plates were reactivated at 110°C for 30 minutes to one hour prior to use The samples were quantitatively applied in one streak on the plate A TLC phospholipid standard consisting of lysolecithin sphingomyelin lecithin and cephalin was also applied The chromatograms were developed with chloroform/methanol/water (65 25 4) The spots were visualized using $I_2$ vapor allowing the plate to stand in the vapor only long enough to produce a light outline of the spots. Iodine could possibly interfere with unsaturated fatty acids Several duplicate samples were chromatogrammed comparing $I_2$ staining to detection with 2 7 dichlorofluorscein The two methods of detection produce identical fatty acid patterns which would indicate minimal if any effect of $I_2$ on the fatty acids Light staining with complete evaporation of the $I_2$ before methylation was still the procedure used as a precaution

*Transmethylation of fatty acids*

Methylation was accomplished according to a modification of Metcalfe (16) Lough (15) and Fulk (5) have reported artifacts associated with the use of boron trifluoride-methanol reagent which affected the results obtained for unsaturated fatty acids The use of 14% $BF_3$-methanol was compared with other reagents such as methanolic HCL methanolic NaOH and $NaOCH_3$ with no significant differences in the results observed Others have studied the boron trihalide methanol reagents and found only time and temperature of reaction to be factors affecting the results (13 17) When certain less pure grades or brands of hexane were used to extract the methyl esters a large peak appeared beyond the 18 carbon fatty acid methyl esters as an artifact If >99 mol% pure hexane was used this peak did not appear

The silica gel containing the lecithin spot was scraped into 20×160 mm culture tubes with teflon lined screw caps Three to five tenths ml of chloroform was added to dissolve the lipids from the silica gel One ml of 14% $BF_3$-methanol reagent was added and the tubes were tightly capped Samples were reacted at 80°C for 15 minutes The methyl esters were extracted three times with 1½ ml hexane The extract was washed once with 1½ ml 0 5 M KCl and twice with 1½ ml water The extract was shaken with a small quantity of anhydrous $Na_2SO_4$ to remove traces of water remaining after washings The hexane extract was decanted and the $Na_2SO_4$ was rinsed with a small portion of hexane which was added to the extract The extract was evaporated to dryness under $N_2$ and quantitatively transferred to a small sample tube and again evaporated to dryness under $N_2$

*Gas liquid chromatography (GLC)*

Samples were redissolved in 10λ hexane λ injected on the chromatography column A Packard Model 7400 GLC was used equipped with a flame ionization detector a 2 m coiled glass column (2 mm inside diameter) The column was packed with 15 DEGS on Chromosorb W(HP) 80/100 mesh The oven was operated isothermally at 180°C with the inlet and detector ovens at 10°C $N_2$ was the carrier gas and run at 15 ml/minute Quantitation is reported as the percentage of the total fatty acid recovered Area of peaks were calculated using the formula height times width at ½ the height

## RESULTS

A small increase in the percentage of saturated fatty acids in amniotic fluid lecithin was observed as the gestational age increased from to 42 weeks (Fig 1a) At 6 to 29 weeks gestation saturated fatty acids comprised 58–70 of the total fatty acids This increased to 77 90 near term Maternal condition (diabetes mellitus Rh sensitization or other) was not associated with

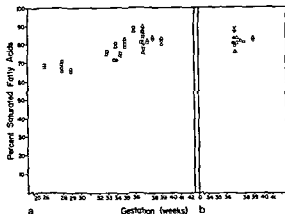



*Fig 1a* Percentage of total fatty acids which constitute all *saturated fatty acids* The samples represent the gestational age △ Diabetic □ Rh neg ○ Other *(b)* Percentage of total fatty acids which constitutes all *saturated fatty acids* The values represent samples which were drawn at delivery or within 7 days prior to delivery △ Diabetic □ Rh neg ○ Other Solid characters represent patients who delivered babies with RDS

any particular pattern of percentage of saturated fatty acids The range of saturated fatty acids in lecithin from amniotic fluid obtained within 7 days of delivery is presented in Fig 1*b* A low percentage of saturated fatty acids in the amniotic

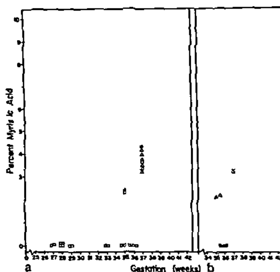



*Fig 2 (a)* Percentage of total fatty acids which constitutes *myristic acid* The samples represent the gestational age △ Diabetic □ Rh neg ○ Other *(b)* Percentage of total fatty acids which constitutes *myristic acid* The values represent samples which were drawn at delivery or within 7 days prior to delivery △ Diabetic □ Rh neg ○ Other Solid characters represent patients who delivered babies with RDS

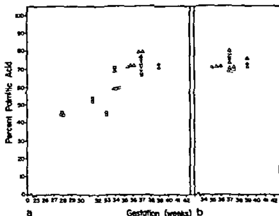



*Fig 3 (a)* Percentage of total fatty acids which constitutes *palmitic acid* The samples represent the gestation age △ Diabetic □ Rh neg ○ Other *(b)* Percentage of total fatty acids which constitutes *palmitic acid* The values present samples which were drawn at delivery or within 7 days prior to delivery △ Diabetic □ Rh neg ○ Other Solid characters represent patients who delivered babies with RDS

fluid lecithin occurred with only one of the five patients who developed RDS

Myristic acid (Fig 2*a*) appeared in only 4 of 15 samples before 32 weeks gestation After 32 weeks 11 of 45 samples contained no myristic acid Of these 5 were associated with infants who developed RDS (Fig 2*b*) All of the samples which contained myristic acid were patients whose babies had no respiratory distress

The concentrations of palmitic acid in amniotic fluid lecithins as a function of gestation are presented in Fig 3*a* Between 26 and 32 weeks gestation palmitic acid comprised 25–55% of the total fatty acids From 32 to 40 weeks gestation this increased to 50–80% The concentration of palmitic acid in amniotic fluid lecithin obtained within one week of delivery are presented in Fig 3*b* Four of the 5 *patients with RDS had* amniotic fluid lecithins with lower percentages of palmitic acid than patients without RDS at comparable gestational age

Stearic acid as a percentage of the total fatty acid composition of the *amniotic fluid lecithins* at different gestations is presented in Fig 4*a* Prior to 32 weeks gestation the range of stearic acid was 16–44% After 36 weeks gestation only 2 of 20 samples contained more than 8% stearic acid *in the amniotic fluid* lecithins The data from fluids obtained within a week of delivery is presented in Fig 4*b* Of these samples all

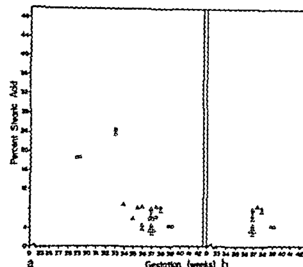


*Fig 4 (a)* Percentage of total fatty acids which constitutes *stearic acid* The samples represent the gestational age Δ Diabetic ☐ Rh neg ○ Other *(b)* Percentage of total fatty acids which constitutes *stearic acid* The values represent samples which were drawn at delivery or within 7 days prior to delivery Δ Diabetic ☐ Rh neg ○ Other *Solid characters represent patients who delivered babies with RDS*

obtained after 34 weeks gestation *five* of the six containing lecithin with more than 12% stearic acid were obtained from mothers whose infants developed RDS

Oleic acid as a percentage of the total fatty acid composition is presented in Fig 5*a* The percentage of oleic acid decreases with gestational age from 16–32% between 26 and 30 weeks to 4–16% after 36 weeks gestation *The* samples obtained within *one* week of delivery are presented in Fig 5*b* The percentage of oleic acid appeared to have no relationship in the presence or absence of RDS The one infant with a high percentage of oleic acid and RDS was the same infant with a low percentage of total saturated fatty acids

A ratio of stearic acid concentration to oleic acid concentration was calculated and correlated to gestational age in Fig 6*a* The ratios ranged from 0 3 to 2 6 Before 34 weeks there were no ratios below 0 8 Ratios from amniotic fluid obtained within 7 days of delivery we presented in Fig 6*b* Two of seven ratios above 1 0 were from patients who delivered babies without RDS Ratios below 1 0 were all from amniotic fluid obtained from patients who delivered babies without RDS

After 34 weeks gestation infants who developed

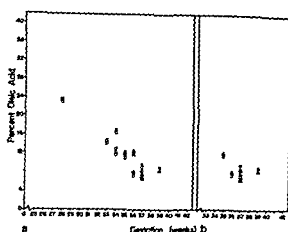


*Fig 5 (a)* Percentage of total fatty acids which constitutes *oleic acid* The samples represent the gestational age Δ Diabetic ☐ Rh neg ○ Other *(b)* Percentage of total fatty acids which constitutes *oleic acid* The values represent samples which were drawn at delivery or within 7 days prior to delivery Δ Diabetic ☐ Rh neg ○ *Other* Solid characters represent patients who delivered babies with RDS

RDS were associated with amniotic fluid containing lecithins which were characterized by higher concentrations of stearic acid no myristic acid and relatively low concentrations of palmitic acid Diabetes mellitus *Rh* sensitization or other maternal problems did not influence the *pattern* of fatty acids from the amniotic fluid lecithins

The fatty acid composition of the amniotic fluid lecithins associated with infants having RDS are presented in Table I As can be noted samples at gestational ages as late as 36–38 weeks had *16 to* 34 5% stearic acid (C 18) and no myristic acid (C 14) The *fatty* acid compositions in

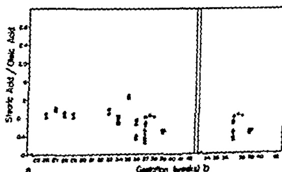


*Fig 6 (a)* Stearic acid to oleic acid ratio *(S/O)* The samples represent gestational age Δ Diabetic ☐ Rh neg ○ Other *(b)* S/O Ratio The values represent samples which were drawn at time of delivery or within 7 days prior to delivery Δ Diabetic ☐ Rh neg ○ Other Solid characters represent patients who delivered babies with RDS

Table I *Fatty acid concentrations of lecithin in amniotic fluid from patients who delivered babies with RDS*

| Patient | Delivery gestation (weeks) | Sample gestation (weeks) | Fatty acid concentration (percent of total fatty acids) Carbon number 14 | 16 | 18 | 18 1 | SA/OA | |
|---|---|---|---|---|---|---|---|---|
| 1 RF | 36 | 36 | 0 | 71 6 | 16 [illegible] | 8 0 | 02 | Mod RDS |
| 2 TG | 36 | 36 | 0 | 28 2 | 34 5 | 26 | 1 32 | RDS died |
| 3 BS | 38 | 38 | 0 | 58 9 | 19 0 | 13 8 | 1 38 | Mod RDS |
| 4 GF | 35 | 27 | 0 | 27 3 | 30 1 | 29 8 | 1 01 | Mild RDS |
| GF | 35 | 8 | 0 | 46 [illegible] | [illegible]3 4 | 21 0 | 1 11 | |
| 5 TJ | 35 5 | 8 | 0 | 44 4 | 6 6 | 16 5 | 1 61 | |
| TJ | 35 5 | 31 5 | 0 | 5 1 | 28 7 | 11 8 | 43 | Mod RDS |
| 6 BN | 37 | 3‿ | 0 | 45 3 | 6 4 | 19 8 | 1 33 | |
| BN | 37 | 37 | 0 | 49 7 | 30 9 | 13 4 | 2 30 | RDS died |
| 7 LT | 36 | 34 | 0 | 58 0 | 2 4 | 13 0 | 1 87 | Mod RDS |
| 8 LAL | 36 | 34 5 | 0 | 49 7 | 25 4 | 14 8 | 1 71 | |
| LAL | 36 | 35 5 | 0 | 52 3 | 25 5 | 14 8 | 1 72 | Severe RDS |

several samples associated with infants without RDS are presented in Table II Only one patient (K S ) delivered prior to 37 weeks gestation

There are some differences in the fatty acid patterns at a given gestational age e g M W and I L J Two patients M W and R H both appear to retain the pattern of high stearic acid low myristic acid through 35 weeks gestation Since they delivered 1½ to 4 weeks after the last sample it is not possible to correlate the pattern on the last sample with the presence or absence of RDS in the baby

Table II *Fatty acid concentrations of amniotic fluid lecithins where more than one samples was obtained representing different gestational ages None of the babies developed RDS*

| Patient | Delivery gestation (weeks) | Sample gestation (weeks) | Fatty acid concentration (percent of total fatty acids) Carbon number 14 | 16 | 18 | 18 1 | SA/OA |
|---|---|---|---|---|---|---|---|
| 1 TB | 37 5 | 36 | 1 0 | 67 3 | 8 5 | 1 5 | 0 68 |
| TB | 37 5 | 37 5 | 2 5 | 71 2 | 8 4 | 9 8 | 0 86 |
| 2 BG | 37 | 36 | 4 0 | 71 6 | 4 6 | 12 0 | 0 38 |
| BG | 37 | 37 | 3 7 | 80 2 | 3 1 | 8 6 | 0 36 |
| 3 MW | 39 | 26 | 0 | 50 9 | 18 1 | 20 6 | 0 88 |
| MW | 39 | 8 | 1 1 | 39 7 | 19 3 | 23 4 | 0 83 |
| MW | 39 | 31 | 0 | 24 3 | 35 7 | 29 0 | 1 23 |
| MW | 39 | 33 | 0 | 45 4 | 23 8 | 19 5 | 1 22 |
| MW | 39 | 35 | 2 3 | 58 2 | 18 1 | 14 2 | 1 27 |
| 4 KS | 34 5 | 27 | 0 | 44 7 | 0 9 | 22 0 | 0 95 |
| KS | 34 5 | 8 | 0 | 44 2 | 21 0 | 23 0 | 0 91 |
| KS | 34 5 | 29 | 0 | 48 1 | 18 8 | 21 2 | 0 98 |
| KS | 34 5 | 33 | 4 0 | 53 5 | 16 9 | 17 3 | 0 98 |
| 5 ILJ | 39 | 29 | 0 | 43 2 | 27 3 | 18 1 | 1 23 |
| ILJ | 39 | 34 | 3 2 | 70 5 | 10 0 | 12 0 | 0 83 |
| ILJ | 39 | 37 | 3 6 | 7 4 | 6 1 | 9 2 | 0 66 |
| 6 RH | 37 5 | 29 | 3 5 | 40 4 | 18 9 | 23 5 | 0 80 |
| RH | 37 5 | 35 | 0 | 8 8 | 32 3 | 8 8 | 1 32 |
| 7 FO | 38 | 33 | 4 6 | 44 9 | 24 4 | 14 4 | 1 69 |
| FO | 38 | 35 | 1 8 | 63 8 | 14 0 | 11 3 | 1 24 |

## DISCUSSION

The fatty acid pattern in lecithins from amniotic fluid have been shown to reflect pulmonary maturity Lecithins containing C 14 and C 16 saturated fatty acids demonstate a lower surface tension than lecithins containing unsaturated fatty acids (7 18) Mature lung lecithin is uniquely dipalmitoyl or palmitoylmyristyl lecithins (6 12) If amniotic fluid lecithin is representative of lung lecithin as we expect an increase should be observed in these saturated fatty acids from amniotic fluid lecithin Two reports recently published show a correlation between palmitic acid concentration and the L/S ratio (1 21) Warren (21) states that a good correlation exists between low palmitic acid concentration and L/S ratio and that palmitic acid concentration separated those samples which were judged immature by L/S ratio Warren s report did not positively identify RDS cases in the illustration she presented

The rate of pulmonary maturation has been related to some maternal problems such as toxemia diabetes mellitus Rh sensitization etc (10) Our data contained too few samples of the various maternal disease categories to make conclusive statements regarding these associations From the data we do have possibly one such correlation could be found In a single case of RDS from a diabetic mother the fatty acid composition was compatible with accelerated maturation This would correspond to Class D–F diabetics according to Gluck et al (10) No other fatty acid patterns were discernible in relation to maternal condition

Amniotic fluid lecithin myristic acid in most instances is low or absent during early gestation (prior to 33 weeks) After 33 weeks we observed low but increasing concentrations of myristic acid (1–8 %) Myristic acid was absent in amniotic fluid lecithins from patients who delivered babies with RDS Arvidson (1) reported a similar increase of amniotic fluid lecithin myristic acid as gestational age increased from 31 weeks Gluck et al (8) found in tracheal aspirates which reflect lung lipids the reverse pattern correlated to gestational age He found appearance of myristic acid about 31 weeks which then decreased near term Arvidson (1) suggested this disagreement may be due to acetone precipitation However in experiments in our laboratory we have been unable to demonstrate any significant differences in the fatty acid patterns of the acetone soluble and acetone insoluble fractions using the volumes of acetone according to Gluck (9)

Palmitic acid composition of amniotic fluid lecithin increased after 33 weeks of gestation When RDS occurred palmitic acid content in the lecithin was below 60 % from samples obtained within one week of delivery Arvidson et al (1) and Warren (21) have previously correlated palmitic acid content with the corresponding L/S ratios Our data is similar to these studies but varied quantitatively Warren et al (21) reported the palmitic acid in millimolar (mM) values of about 0 07 mM palmitic acid as the concentration that separates maturity and immaturity of the lung When we calculated mM values from our absolute lecithin quantitation and the palmitic acid percentages of total fatty acids we obtained 0 007 mM palmitic acid as the point of separation A possible explanation for the discrepancy might be due to differences in methods of obtaining the quantitative mM values If Warren used internal standardization in the gas chromatography the values reported may be more accurate The general pattern of L/S ratio plotted against palmitic acid concentration in our data was more scattered than that of Warren

As gestation progressed the stearic acid content in amniotic fluid lecithin decreased as a percentage of the total fatty acids The fluid from patients who delivered babies with RDS had abnormally high stearic acid content (>1[illegible] %) A correlation between stearic acid and linoleic acid has been noted by others (4) Since the stearic acid peak runs adjacent to the oleic acid peak in the gas chromatogram we noted a correlation between these two fatty acids We found in those cases which involved RDS the stearic acid peak was greater than the oleic acid peak which was contrary to the pattern usually seen in amniotic fluid lecithins When the ratio of percent stearic acid to percent oleic acid was calculated ratios greater than 1 0 were from patients delivering babies with RDS and those less than 1 0 delivered babies without RDS This increase in stearic acid content indicates an increase of stearic acid esterified to the lecithin in place of the normal palmitic or myristic acids and would also increase the surface tension above the corresponding palmitic and myristic acid species

Lung maturation is indicated by a particular pattern of the amniotic fluid lecithin fatty acids An amniotic fluid which contains lecithin with a significant myristic acid (>1%) high palmitic acid (>60%) and low stearic acid (<16%) or low SA/OA ratio (<1 0) could be considered mature If the baby was born with these conditions present at delivery we found no complications of respiration When the reverse pattern was observed (Pa< 60% MA<1 0% SA>16% or SA/OA>1 0) RDS frequently occurred if the the baby was born at that time


## ACKNOWLEDGEMENTS

We wish to thank the staff of the Pediatric Research Institute Rikshospitalet for their support We are especially grateful for the assistance of Mr Leif Skjelkvåle The study has been supported in part by The John A Hartford Foundation New York N Y S N G John and Mary Markle Scholar


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*Submitted for publication August 13 1973*

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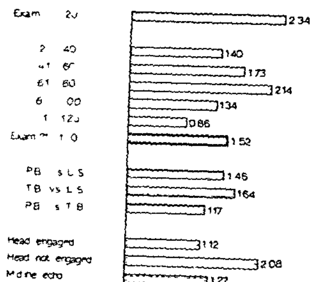



*Fig. 1.* Mean observer errors in duplicate measurements of the fetal biparietal diameter. Further explanations see text.

was used for orientation, while the actual measurement was done on the A scan beam with the help of a millimeter scale on the oscilloscope screen. As a rule the measurements were performed from transversal B scan sections. If the right plane for measurement was not found readily, the apparatus was moved, and a longitudinal scan obtained. The time taken for the single examination varied, with means of 4.9 min (L. S.), 3.8 min (P. B.) and 3.5 min (T. B.).

## RESULTS

The results are shown graphically in Fig. 1. The horizontal bars show the standard deviations of the differences of duplicate measurements. The upper bar, representing the first 20 examinations, shows a fairly high degree of observer error. This represents the end of L. S.'s training period. The following five series of 20 consecutive examinations each (examinations no. 21–120), representing 71 women with gestational age ranging from 183–308 days, is thought to give a fair impression of the true observer error. This error ranges from 0.86 to 2.14 mm, and shows no clear trend with time. The over all error of these last 100 measurements was 1.52 mm, which means that 95% of the differences between two independent observer measurements were below 3.04 mm and 68% below 1.52 mm. The next two bars show the result for each obstetrician (P. B. and T. B.) tested separately against those of L. S., the errors being of the same magnitude, 1.46 mm and 1.64 mm respectively. The next bar represents a series of 20 duplicate measurements, which were all performed by P. B. and T. B. This error, 1.17 mm, is low, which indicates that accuracy of measurements will increase with increasing experience. The following two bars show that the error was about twice as high when the fetal head was not engaged as when it was engaged (2.08 mm against 1.12 mm). Engagement of the fetal head is here used in its widest sense, meaning that the head is partly fixed in the pelvic inlet but that the biparietal diameter is still above the linea terminalis, permitting the measurement to be made. Engagement of the fetal head facilitated the measurements greatly, since head movements were then slight or absent during the examinations. On the other hand, freely moving heads made the examinations more difficult, since they might disappear completely from the field of examination during adjustment of the apparatus, particularly in breech presentation, hence the large error. The bottom bar shows that when both observers clearly observed the midline echo, the error was low (1.27 mm).

The next question was whether the observer differences were due to a systematic error on the part of one examiner. The distribution shown in Fig. 2 represents the differences between L. S.'s measurements and those of P. B. and T. B. when the differences were signed as positive or negative. Statistical analysis showed that the mean of this distribution did not differ significantly from 0. The same holds true when the differences between

*Table I. Distribution of positive and negative differences in 100 consecutive duplicate measurements of the fetal biparietal diameter (examinations 21–120)*

| | L. S. vs P. B. | L. S. vs T. B. | Total |
|---|---|---|---|
| No. of measurements with positive difference | 22 | 14 | 36 |
| No. of measurements with negative difference | 18 | 10 | 28 |
| No. of measurements with equal results | 22 | 14 | 36 |

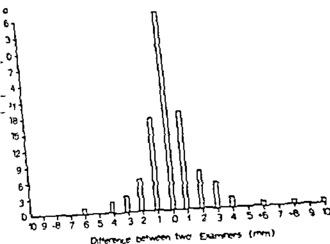



Fig 3 Distribution of positive and negative differences in 6[illegible] duplicate cephalometric examinations performed by L S and P B and 38 performed by L S and T B

L S and P B and between L S and T B were analysed separately (Table I) Positive and negative differences were about equally distributed in both instances It may be concluded that the observer error occurred at random and that it was not caused by a systematic interpretation error on the part of one observer

## DISCUSSION

The observer error in fetal cephalometry may be due to several factors The relative importance of each factor may vary from case to case Firstly the design of the apparatus particularly its movability and the mode of obtaining the correct measurement on the oscilloscope is of some importance The apparatus we used was easy to angulate but difficult to move from a transverse to a longitudinal position The actual measurement was read on a millimeter scale by counting A built in caliper system with display of the measurement by numerals might have been an improvement Secondly the position and the movability of the fetal head is of great importance which is evident from the results With a freely movable fetal head particularly in breech presentation it was often difficult to obtain the correct angulation of the transducer and the fetal head might move while the final adjustments were made In this investigation each examiner worked alone The help of an assistant *might* have improved the accuracy of the measurements Thirdly the ability of the examiner is an important point The examiner must be thoroughly familiar with the apparatus but it is doubtful whether an important gain in diagnostic accuracy can be expected with increasing technical experience once a certain level is reached Our results showed no clear improvement in observer error with time On the other hand experience in obstetrical examinations should ensure less observer error Obviously the maximum accuracy and the least observer error will be achieved if the examiner always adheres strictly to the rules laid down by Campbell (1) but everyone who has tried the method knows that technical difficulties do arise from time to time in which cases a greater observer error must be expected It is possible that the magnitude of observer error which we have reported here will seem unacceptably high to other workers in the field However every ultrasonic unit should have an estimate of its own observer error just as every laboratory should know the precision of its own analyses

Awareness of the observer error in ultrasonic fetal cephalometry is very important when growth indices are assessed In cases of suspected intrauterine growth retardation the growth index has been shown to be a better prognostic sign than the urinary estrogen assays (4) To minimize the observer error in such cases the biparietal diameter should be measured frequently and ideally by two examiners *each time*

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*Submitted for publication Sept 17 1973*

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Acta Obstet Gynec Scand 54 45–48 1975

# SHORT TERM CHANGES IN STILLBIRTH RATES IN SWEDEN 1965–1971

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*Abstract* An investigation was made of short time changes in stillbirth rates in Sweden 1965–1971 The data is based on official statistics from the whole of Sweden The material has been divided into three maternal age groups less than 30 years 30–34 years and 35 years or more Division by parity was made in five groups parity 1 2 3 4 and 5 or higher (5+) The stillbirth rates in most groups show an approximately steady level or a very slight decrease during the years under study However the parae 1 ? and 5+ in the age group 35 years or over and para 5+ in age group 30–34 years deviate from this pattern There is a decrease to 1969 followed by an increase To test the apparent increase in stillbirth risk during the period 1969–1971 for high risk groups a $\chi^2$ analysis was made For parae 1 4 and 5+ aged 35 years or more a significant heterogeneity between years was found This increase cannot be explained by a shift in age distribution within the age group ≥35 years Exogenous factors may be involved and further studies are planned in order to solve the problem

In an earlier study of stillbirths in Sweden (7) variations in overall stillbirth rates were noticed during the relatively short period 1965 to 1971 It was observed that the changes were most marked where maternal age was over 35 years *The risk of stillbirth is* closely related to maternal age and parity (cf 2 3 4 9 10 11) and maximum risk occurs with high—and very low—maternal age and with parity 1 or parity 4 or higher During the period studied marked changes occurred in family planning opportunities mainly due to the introduction of oral contraceptives and intra uterine devices Such factors would tend to reduce the number of pregnancies in older women and in those of high parity This would affect the overall stillbirth rates

Factors other than age and parity are of obvious importance in relation to stillbirth risk Barron (1) reviewed some aspects of the epidemiology of human reproduction and pointed out that the gradual decrease in perinatal mortality seen in England and Wales during the past few decades could be due to improved antenatal care and general social improvements Other exogenous factors can also be of importance e g maternal infections toxic chemicals in the environment and smoking (5) In the present study an effort has been made to further analyze the changes in stillbirth rates which were noted in the earlier report (7)

## MATERIAL

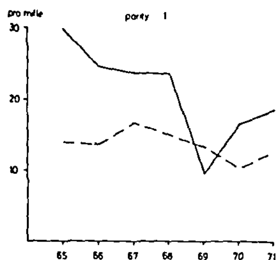
Information was obtained from the official Swedish statistics National Central Bureau of Statistics (6) Information concerning parity and maternal age distribution could be obtained only for live births and stillbirths in present marriages contracted 1950 or later—but children born within the marriages but before 1950 are also *included There is* thus a slight discrepancy between true biological parity and the parity concept used in this study but it is of little quantitative importance

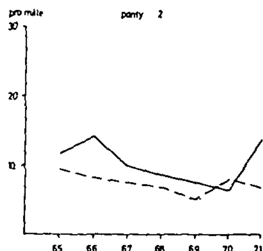
The material was divided into three maternal age groups less than 30 years 30–34 years and 35 years or more Division by parity was made in five groups parity 1 ? 3 4 and 5 or higher For each subgroup the stillbirth rate per total number of births was calculated Possible differences between groups were tested with $\chi^2$ tests for heterogeneity

## RESULTS

Fig 1 presents the stillbirth rates by calendar years for different maternal age groups and parities The subgroup age below 30 years and parity class 5 or more is so small that comparison of different years is not meaningful In this subgroup of 2 222 births 20 stillbirths were recorded—an incidence of 9 0 pro mille

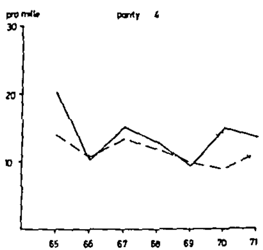
Most graphs in Fig 1 show an approximately

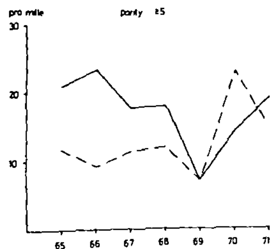









< 30 years
30–34 years
≥ 35 years

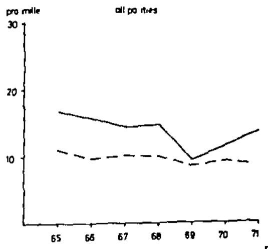



*Fig 1* Stillbirth rate as pro mille at different parities and maternal age groups 1965–1971

Table 1 *$\chi^2$-analysis of heterogeneity between calendar years within age and parity classes*
The $\chi^2$ value for each subgroup is based on 6 d f

| Age group | Parity groups 1 | 2 | 3 | 4 | ≥5 | $\Sigma\chi^2$ |
|---|---|---|---|---|---|---|
| ≥35 | 18 1 | 13 5* | 8 8 | 7 5 | 8 7 | 56 5 |
| 30–34 | 5 3 | 10 1 | 10 2 | 2 2 | 7 1 | 34 9 |
| <30 | 12 0 | 14 9 | 5 2 | 2 4 | – | 34 5 |
| $\Sigma\chi^2$ | 35 4 | 38 5 | 24 2 | 12 1 | 15 8 | 125 9 |

horizontal level or a very slight decrease with calendar year Four groups apparently deviate from this pattern parae 1 2 and 5 or over in age group 35 or over and parae 5 or over in age group 30–34 In these groups there is a more or less marked decrease up to 1969 followed by an increase to 1971 These changes are sufficient to give a similar form to the graph for all parities age 35 or over

The statistical analysis of possible differences in incidence between different years within subgroups is shown in Table 1 Within subgroups a significant heterogeneity between years is found only for primiparae aged 35 or over The changes for parae 2 aged 35 and over or below 30 are possibly significant The dominating change in risk figures during the period of study is a decrease from high initial values to an apparent minimum around 1969 It was therefore thought to be of interest to see if the apparent increase during the period 1969–1971 is significant for the high risk groups The total stillbirth incidence for parae 1 4 and ≥5 aged 35 and over was compared during the three years in 1969 it was 10 2 (3 664 births) in 1970 it was 16 7 (3 524 births) and in 1971 it was 16 9 (3 301 births) pro mille The three rates are significantly different in a $\chi^2$ test for heterogeneity ($\chi^2$=7 1 0 05>$P$>0 01) For primiparae changing age distribution within the age group 35 years or over could explain the apparent rise if a shift occurs within the age group and if the stillbirth risk increases steeply with age above 35 years The age distribution within smaller age groups was therefore studied 35–37 38–40 41–43 44–49 years

Fig 2 presents the percentage distribution of these four subgroups—no marked changes occurred during the observation period and no differences exists between the years 1969 1970 1971 $\chi^2$= 4 6 at 6 d f 0 6>$P$>0 5 Thus the increase in stillbirth rate in primiparae aged 35 years or over cannot be explained by age shifts within the sub group

## DISCUSSION

Changes previously observed for the general stillbirth rate in Sweden during the period 1965–1971 when analysed by age and parity are small and not significant for most age and parity classes The only very marked changes occurred in primiparae aged 35 years or over within this group there is a marked decline in stillbirth rate up to 1969 followed by an increase The same type of biphasic graph is seen also for parae 2 and 5 or over who are aged 35 or over within the same age class and for parae 5 or over aged 30–34 years

From the USA it has been reported that teenage mothers are a high risk group (4) The majority of such women are likely to be primigravidae The stillbirth rate of this group does not differ from that of all women ≤30 years and a $\chi^2$ analysis of heterogeneity is not significant It is concluded that in the Swedish population the teenage mothers as a total group are not a high risk group

Primiparae aged 35 years or over showed marked changes in stillbirth risk during the period of study The possibility that this could be due to changes in the age composition of the group was entertained However an analysis of the actual age distribution shows that it is quite constant during the years 1969–1971 inclusive

It is thus difficult to explain the changes in stillbirth rate in the high risk groups by changes

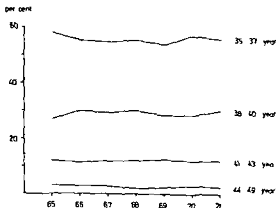



*Fig 2* Maternal age distribution within age group ≥35 years 1965–1971

in the two most well known correlates to stillbirth rate age and parity Barron (1) stressed that social and medical factors may influence stillbirth rate In this connection one social factor could obviously influence the stillbirth rates registered namely immigration Immigration to Sweden especially from South Europe declined between 1965–1968 and then rose again to a maximum in 1970 In 1966 approximately 16 000 persons immigrated from South Europe in 1968 5000 and in 1970 17 000 These differences are thus marginal but among immigrant parturients approximately 30% are 35 years or older (6) compared to 5–7% of parturients of Swedish stock It is also known (8) that a great part of the immigrants at least from South Europe to a large extent live in social misery in Sweden partly due to lack of knowledge of the Swedish language which probably gives them difficulties in getting work and also information on social securities e g antenatal care These factors should affect stillbirth rates in the directions observed a reduction followed by a secondary increase On the other hand quantitatively births among immigrants above 35 years are few in 1965 they comprised only 0 7% of all births in this age group and in 1971 they had increased to 2% Even then they cannot explain the increase noted especially in primiparae

Other factors of social or medical nature can affect the total population at risk but it is difficult to suggest suitable candidates for such factors Exogenous factors can be of importance The continual supervision of stillbirth rates especially within high risk groups seems worth while in order to identify further changes perhaps of a more drastic nature

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Submitted for publication Oct 5 1973

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Acta Obstet Gynec Scand 54 49–55 1975

# THE COAGULATION SYSTEM DURING CAESAREAN SECTION

*Coagulation Fibrinolysis and Hormonal Levels in Peripheral and Uterine Venous Blood during Caesarean Section*

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*Abstract* Some parameters of blood coagulation and fibrinolysis have been studied in peripheral and uterine blood during elective Caesarean section in ten women The levels of estradiol and progesterone were also studied Before the operation there were lower levels of fibrinolytic inhibitors in uterine blood than in peripheral blood Therefore the inhibitors present in the placenta seem to be restricted to the fetal circulation During the course of the operation there was a shortening of the activated partial thromboplastin time APTT in peripheral and uterine blood The number of platelets and the factor VIII activity increased in uterine blood and the values in peripheral blood also tended to increase These changes speak in favour of a general clot promoting tendency after placental separation After placental separation there was a lower plasminogen level in uterine blood compared to peripheral blood which supports a hypothesis of local uterine fibrinolysis after placental separation The level of estradiol and progesterone in peripheral blood did not change during the operation In uterine blood the concentrations of both hormones tended to decrease No correlation was found between the levels of the hormones and the parameters of coagulation and fibrinolysis

During pregnancy there is a markedly decreased fibrinolytic activity in peripheral blood (5 7 9 35 37) This decrease has tentatively been explained by a decreased release and/or synthesis of activators of fibrinolysis in the vessel walls (3 9 35 41) However some authors have reported increased amounts of inhibitors of fibrinolysis in serum from pregnant women (14 26 31) Other authors have not been able to demonstrate such an increase (5 9 10 22) Several tissue inhibitors of fibrinolysis have been isolated from the placenta (2 20 26 27 40) and it has been shown that the decrease in fibrinolytic activity is dependent on the presence of the placenta and not of the fetus (3) The placenta may influence fibrinolysis directly by means of inhibitors located in the placenta but it may also be an indirect influence via its hormonal secretion From the experimental design of previous studies it has not been possible to determine whether estrogens influence the synthesis a comparatively slow process or the release of plasminogen activators in the vessel walls which might be a faster process (3)

In previous work fibrinogen fibrin degradation products FDP were shown to increase rapidly after separation of the placenta The increase was more marked in uterine venous blood indicating that the uterus was the source of the FDP (18) Additional information has been obtained by studies on blood draining the feto placental unit compared to peripheral venous blood Differences between FDP levels in uterine venous and peripheral venous blood were greater when there had been uterine contractions One can assume that changes in parameters other than FDP would also be greater in emergency than in elective Caesarean sections

However Caesarean sections are often made in emergency situations which make blood sampling difficult and hazardous Therefore only women who were subjected to an elective Caesarean section were studied in the present work The aim of the study is to extend the previous investigations and to

1) compare uterine and peripheral venous blood in term pregnancies with regard to blood coagulation and fibrinolysis

2) study the effect of the removal of the fetus and the placenta on coagulation and fibrinolysis and

3) study the possible correlation between coagulation—fibrinolysis and two placental hormones estradiol and progesterone with special attention to the fibrinolytic activators and inhibitors

## CLINICAL MATERIAL

The clinical material consists of ten women at term The indication for Caesarean section was a contracted pelvis in nine cases The tenth woman was in the 37th week and had a toxemia of pregnancy The blood pressure was 160/100 mmHg and the urinary protein was 2 g/l *The reason for the operation was a day to-day* decreasing urinary estrogen secretion

Premedication included single doses of atropin 0 3 mg together with diazepam (Valium® F Hoffman La Roche & Co AG Basle Switzerland) 10 mg or scopolamine 0 5 mg Anesthesia was induced by enhexymal sodium (Evipan® Bayer AG Leverkusen West Germany) and suxamethanium chloride (Celocurin® Vitrum AB Stockholm Sweden) and maintained with nitrous oxide and oxygen The relaxant used was d tubocurarine chloride or *alcuronium chloride (Alloferin® F Hoffman* La Roche & Co AG Basle Switzerland) Oxytocin (Syntocinon®) 10 IU and methyl-ergometrin (Methergin®) 0 2 mg both from Sandoz AG Basle Switzerland was given as soon as the placenta was removed

## METHODS

### *Collection of blood*

Blood samples were obtained simultaneously from a *uterine vein and from an antecubital vein on three oc*casions 1) immediately before the incision into the uterine wall 2) 5 minutes after removal of the placenta and 3) 15–20 minutes after removal of the placenta The blood from the uterine vein was drawn into a glass syringe and transferred to plastic tubes where it was mixed with *1/10* its volume of 0 1 M trisodium citrate The peripheral blood was allowed to flow directly into the tubes The tube for fibrinogen determination contained lysine ethylester to prevent fibrinogenolysis The *tubes were placed on ice until the blood* was analyzed usually within half an hour The concentrations of the hormones and of antithrombin III $\alpha_1$ antitrypsin and $\alpha_2$ macroglobulin were determined on samples that had been deep-frozen

### *Analyses of blood coagulation*

Platelets were counted in a Celloscope (Ljungberg & Co Stockholm Sweden)

Activated partial thromboplastin time APTT was carried out with the commercial reagent Platelin plus activator General Diagnostics Division Warner Lambert Co Morris Plains USA

Fibrinogen was determined according to Morrison s syneresis method as modified by Blomback (8)

Factor V was determined according to the method of Aas (1)

Factor II–VII–X activity was determined with the commercial reagent Simplastin A (29)

Table I *Results of the coagulation system assays in peripheral blood (P) and uterine blood (U) before placental separation (0) five and fifteen minutes after placental separation*

Results are given as mean±S E

| | 0 | 5 | 15 | *n* | *sign* |
|---|---|---|---|---|---|
| *Platelets* (×10³/mm³) | | | | | |
| *P* | 193±15 | 206±10 | 199±11 | 9 | n s |
| *U* | 184±12 | 205±11 | 199±12 | 9 | sign |
| *P U* | 9±11 | 1±6 | 0±4 | | |
| *n* | 10 | 10 | 8 | | |
| sign | n s | n s | n s | | |
| APTT (seconds) | | | | | |
| *P* | 30 8±1 3 | 28 9±1 3 | 28 5±1 5 | 10 | sign |
| *U* | 31 9±1 | 30 3±1 5 | 29 3±1 8 | 9 | sign |
| *P U* | −1 1±0 6 | −1 4±0 7 | −0 8±0 6 | | |
| *n* | 10 | 10 | 9 | | |
| sign | n s | n s | n s | | |
| Fibrinogen (g/l) | | | | | |
| *P* | 4 58±0 30 | 4 25±0 22 | 4 25±0 21 | 10 | n s |
| *U* | 4 25±0 21 | 4 10±0 20 | 4 05±0 22 | 9 | n s |
| *P U* | *0 33±0 19* | *0 15±0 06* | *0 20±0 07* | | |
| *n* | 10 | 10 | 9 | | |
| sign | n s | sign | sign | | |
| Factor V (% of normal) | | | | | |
| *P* | 105±21 | 103±21 | 97±21 | 6 | n s |
| *U* | 96±14 | 99±21 | 99±19 | 6 | n s |
| *P U* | 9±11 | 4±6 | −2± | | |
| *n* | 6 | 6 | 6 | | |
| sign | n s | n s | n s | | |
| Factor II–VII–X (% of normal) | | | | | |
| *P* | 179±12 | 166±10 | 170±11 | 10 | n s |
| *U* | 158±8 | 165±9 | 165±12 | 9 | n s |
| *P U* | 21±14 | 1±6 | 5±6 | | |
| *n* | 10 | 10 | 9 | | |
| sign | n s | n s | n s | | |
| Factor VIII (% of normal) | | | | | |
| *P* | 232±34 | 274±35 | 272±36 | 10 | n s |
| *U* | 186±21 | 215±19 | 214±21 | 9 | sign |
| *P U* | 46±21 | 59±22 | 58±26 | | |
| *n* | 10 | 10 | 9 | | |
| sign | n s | sign | n s | | |
| *Antithrombin III* (% of normal) | | | | | |
| *P* | 99 7±4 3 | 100 4±7 2 | 100 8±10 0 | 7 | n s |
| *U* | 95 2±6 7 | 101 2±8 0 | 100 5±8 9 | 9 | n s |
| *P U* | 4 5±5 9 | −0 8±6 0 | 0 3±3 3 | | |
| *n* | 9 | 9 | 8 | | |
| sign | n s | n s | n s | | |

Antithrombin III was determined with immunodiffusion (33) The antiserum was from Behringwerke AG Marburg Lahn West Germany

### *Analyses of the fibrinolytic system*

Plasminogen was determined according to Berg et al (6)

Whole plasma clot lysis was determined according to Korsan Bengtsen et al (30)

Fibrinolytic activity on plasminogen free fibrin plates was determined according to Ygge (42)

Table II *Results of the fibrinolytic system assays in peripheral blood (P) and uterine blood (U) before placental separation (0) five and fifteen minutes after placental separation*

Results are given as mean±S E

| | *0* | *5* | *15* | *n* | sign |
|---|---|---|---|---|---|
| Plasminogen (casein units/ml) | | | | | |
| *P* | 0 6±1 2 | 19 9±0 7 | 19 0±1 1 | 9 | n s |
| *U* | 18 4±0 8 | 18 4±1 0 | 18 1±0 7 | 8 | n s |
| *P U* | 2 2±1 1 | 1 5±0 6 | 0 9±0 7 | | |
| *n* | 9 | 9 | 8 | | |
| sign | n s | sign | n s | | |
| Whole plasma clot lysis (% lysed fibrinogen) | | | | | |
| *P* | 6 9±3 6 | 3 3±1 8 | 8 8±6 2 | 10 | n s |
| *U* | 14 4±10 0 | 25 3±12 6 | 20 0±11 1 | 9 | n s |
| *P U* | −7 5±10 4 | −2 0±12 0 | −11 2±6 8 | | |
| *n* | 10 | 10 | 9 | | |
| sign | n s | n s | n s | | |
| Fibrinolytic activity on plasminogen free plate (mm²) | | | | | |
| *P* | 206±8 | 204±9 | 201±10 | 10 | n s |
| *U* | 212±10 | 220±15 | 210±19 | 9 | n s |
| *P U* | −6±6 | −16±10 | −9±10 | | |
| *n* | 10 | 10 | 10 | | |
| sign | n s | n s | n s | | |
| Fibrinolytic activity on plasminogen rich plate (mm ) | | | | | |
| *P* | 127±12 | 136±15 | 145±24 | 8 | n s |
| *U* | 152±22 | 181±40 | 169±12 | 7 | n s |
| *P U* | −25±15 | −45±33 | −24±12 | | |
| n | 9 | 8 | 8 | | |
| *sign* | *n* s | n s | n s | | |
| $\alpha_1$ antitrypsin (% of normal) | | | | | |
| *P* | 136 4±10 9 | 141 6±10 6 | 132 4±12 4 | 8 | *n s* |
| *U* | 120 8±13 2 | 136 5±9 7 | 123 0±7 4 | 8 | n s |
| *P U* | 15 6±5 0 | 5 1±5 5 | 9 4±8 9 | | |
| n | 10 | 8 | 8 | | |
| sign | sign | n s | n s | | |
| $\alpha_2$-macroglobulin (% of normal) | | | | | |
| *P* | 128 6±12 0 | 116 2±10 4 | 123 0±12 4 | 9 | n s |
| *U* | 118 1±14 2 | 121 7±11 9 | 117 9±12 0 | 9 | n s |
| *P U* | 10 5±11 5 | −5 5±6 1 | 5 1±4 0 | | |
| *n* | 10 | 9 | 9 | | |
| sign | n s | n s | n s | | |
| Total antifibrinolytic activity (% of normal) | | | | | |
| *P* | 137 3±6 4 | | 139 3±6 0 | 9 | n s |
| *U* | 106 0±6 0 | | 119 3±9 0 | 9 | n s |
| *P U* | 31 3±9 0 | | 20 0±8 8 | | |
| *n* | 9 | | 9 | | |
| *sign* | *sign* | | n s | | |

Fibrinolytic activity on plasminogen rich fibrin plates was determined according to Petruson (36)

Total antifibrinolytic activity was determined according to Ygge et al (43)

$\alpha$ antitrypsin and $\alpha_2$ macroglobulin were determined by immunodiffusion (33) The antisera were from Behringwerke AG Marburg Lahn West Germany

*Hormone analyses*

The levels of estradiol were measured by a radioimmunoassay technique (16) The levels of progesterone were determined using a protein binding technique (25) The determinations were made by Dr Elof D B Johansson Department of Obstetrics and Gynecology University Hospital Uppsala Sweden

*Statistical methods*

The changes during the operation were analyzed by a two-way analysis of variance with Scheffe s method of multiple comparison Student s *t* test was used for the paired comparison between uterine and peripheral blood The level of significance was set at 0 05

## RESULTS

*Analyses of the coagulation system*

The results of the analyses of the coagulation system (Mean values±S E ) are given in Table I During the operation the platelets in uterine blood increased whereas in peripheral blood no significant changes were recorded However there were no significant differences between peripheral and uterine blood at any sampling time There was a progressive shortening of APTT after placental separation The shortening occurred sooner in peripheral blood in which differences between the original value and those obtained both at 5 and at 15 after placental removal were statistically significant The APTT in uterine blood at 15 after placental separation was significantly shorter than the preincision value Although APTT in peripheral blood tended to be shorter than in uterine blood at all sampling times no significant differences were found The fibrinogen concentration tended to decrease in both uterine and peripheral blood but the decrease was not statistically significant The fibrinogen concentration was lower in uterine blood than in peripheral blood and the difference was found to be statistically significant after 5 and 15 minutes The levels of factor II–VII–X and of factor V showed no changes during the operation in either uterine or peripheral blood The same activities were found in uterine as in peripheral blood

The level of factor VIII increased during the operation and the difference between the preincision value and that obtained at 15 minutes in uterine blood was statistically significant There was a higher factor VIII level in peripheral blood than in uterine blood at all sampling times and at 5 minutes after placental separation this difference was significant

No changes were observed regarding antithrombin III activity after placental separation in either

3) study the possible correlation between coagulation—fibrinolysis and two placental hormones estradiol and progesterone with special attention to the fibrinolytic activators and inhibitors

## CLINICAL MATERIAL

The clinical material consists of ten women at term The indication for Caesarean section was a contracted pelvis in nine cases The tenth woman was in the 37th week and had a toxemia of pregnancy The blood pressure was 160/100 mmHg and the urinary protein was 2 g/l The reason for the operation was a day to-day decreasing urinary estrogen secretion

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## METHODS

*Collection of blood*

Blood samples were obtained simultaneously from a uterine vein and from an antecubital vein on three occasions 1) immediately before the incision into the uterine wall 2) 5 minutes after removal of the placenta and 3) 15–20 minutes after removal of the placenta The blood from the uterine vein was drawn into a glass syringe and transferred to plastic tubes where it was mixed with 1/10 its volume of 0 1 M trisodium citrate The peripheral blood was allowed to flow directly into the tubes The tube for fibrinogen determination contained lysine ethylester to prevent fibrinogenolysis The tubes were placed on ice until the blood was analyzed usually within half an hour The concentrations of the hormones and of antithrombin III α antitrypsin and $\alpha_2$ macroglobulin were determined on samples that had been deep-frozen

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Factor V was determined according to the method of Aas (1)

Factor II–VII–X activity was determined with the commercial reagent Simplastin A (29)

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| *U* | 184±12 | 205±11 | 199±12 | 9 | sign |
| *P U* | 9±11 | 1±6 | 0±4 | | |
| *n* | 10 | 10 | 8 | | |
| sign | n s | n s | n s | | |
| APTT (seconds) | | | | | |
| *P* | 30 8±1 3 | 28 9±1 3 | 28 5±1.5 | 10 | sign |
| *U* | 31 9±1 | 30 3±1 5 | 29 3±1 8 | 9 | sign |
| *P U* | −1 1±0 6 | −1 4±0 7 | −0 8±0 6 | | |
| *n* | 10 | 10 | 9 | | |
| sign | n s | n s | n s | | |
| Fibrinogen (g/l) | | | | | |
| *P* | 4 58±0 30 | 4 25±0 22 | 4 25±0 21 | 10 | n s |
| *U* | 4 25±0 21 | 4 10±0 20 | 4 05±0 22 | 9 | n s |
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| *n* | 10 | 10 | 9 | | |
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| Factor V (% of normal) | | | | | |
| *P* | 105±21 | 103±21 | 97±21 | 6 | n s |
| *U* | 96±14 | 99±21 | 99±19 | 6 | n s |
| *P U* | 9±11 | 4±6 | −2± | | |
| *n* | 6 | 6 | 6 | | |
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| *P* | 179±12 | 166±10 | 170±11 | 10 | n s |
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| *n* | 10 | 10 | 9 | | |
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| *U* | 186±21 | 215±19 | 214±21 | 9 | sign |
| *P U* | 46±21 | 59±22 | 58±26 | | |
| *n* | 10 | 10 | 9 | | |
| sign | n s | sign | n s | | |
| Antithrombin III (% of normal) | | | | | |
| *P* | 99 7±4 3 | 100 4±7 2 | 100 8±10 0 | 7 | n s |
| *U* | 95 2±6 7 | 101 2±8 0 | 100 5±8 9 | 9 | n s |
| *P U* | 4 5±5 9 | −0 8±6 0 | 0 3±3 3 | | |
| *n* | 9 | 9 | 8 | | |
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Antithrombin III was determined with immunodiffusion (33) The antiserum was from Behringwerke AG Marburg Lahn West Germany

*Analyses of the fibrinolytic system*

Plasminogen was determined according to Berg et al (6)

Whole plasma clot lysis was determined according to Korsan-Bengtsen et al (30)

Fibrinolytic activity on plasminogen free fibrin plates was determined according to Ygge (42)

plastic material can enter the maternal circulation following placental separation or injury (15) The increase in factor VIII activity might be due to a non specific clot promoting substance but might also be due to a real increase in factor VIII originating from spleen contraction (34) However Oxytocin given together with methylergometrine after placental expulsion does not influence the splenic volume or its capillary resistance (4) methylergometrine alone has not been studied in this respect The increase of platelets also suggests the existence of a platelet pool It is notable however that the changes are more marked in the uterine blood than in the peripheral but this might be accidental Bonnar et al (13) reported normal factor IX activity together with increased factor VIII activity after placental removal and drew the conclusion that the increase in factor VIII activity was not due to a non-specific clot accelerator In our opinion this cannot be excluded

The fibrinogen concentration is higher in peripheral than in uterine blood and after placental separation this difference is statistically significant The uterine cavity is very rapidly covered by a fibrin mesh after removal of the placenta (32) The amount of fibrinogen deposited has been estimated as 5–10% of the total fibrinogen content (32) This is in line with the findings in the present study The decrease in the plasma fibrinogen concentration is not statistically significant in this material probably due to the small number of subjects studied However there is a definite tendency to decreasing values A decrease in fibrinogen concentration at normal delivery has been described by several authors (10 28 42) Kleiner et al (28) found reduced levels of fibrinogen two hours after elective Caesarean section Bonnar et al (13) also found a decrease in fibrinogen concentration but not before 2–4 hours after the operation

The plasminogen levels were lower in uterine than in peripheral blood and the differences were statistically significant five minutes after removal of the placenta This supports the concept of a local uterine fibrinolysis This is also in line with the higher levels of fibrinogen fibrin degradation products FDP found in uterine blood compared to peripheral blood after placental expulsion (18)

There were no significant changes in fibrinolytic activity during the operation in this study This is at variance with Bonnar et al (13) who found increased fibrinolytic activity in uterine blood during placental separation Woodfield et al (41) who also used the euglobulin lysis time technique found in peripheral blood a return to normal non pregnant values within less than one hour after a Caesarean section Kleiner et al (28) found the increase in peripheral blood fibrinolytic activity to take place 30–60 minutes after a Caesarean section It may be concluded that the blood sampling was done too soon after delivery to detect an increased fibrinolytic activity in peripheral blood Differences in fibrinolytic activity of the uterine venous blood between those reported in the present study and in the previous study (13) might be explained on methodological basis However there was an obvious trend towards higher fibrinolytic activity in the uterine blood also in the present study in all three assay systems there thus was a higher activity in the uterine blood at all sampling times but the uteroperipheral difference did in no case reach significant levels

It would be of interest to study the fibrinolytic system in uterine blood when there have been uterine contractions The possible hypoxia during labour might cause the release of plasminogen activators from the myometrium (39) Considerably higher FDP levels have been found in uterine blood during Caesarean sections carried out during labour compared with elective Caesarean sections (18)

The level of fibrinolytic inhibitors in blood draining the uterus has not been studied systematically previously Beller et al (5) measured some inhibitors but the blood sampling was done only once before delivery of the child They found no differences between ovarian blood and peripheral blood Bonnar et al (13) measured inhibitors to urokinase induced lysis and found no changes during a Caesarean section and no differences between uterine and peripheral blood One would anticipate higher antifibrinolytic activity in blood draining the placenta as fibrinolytic inhibitors have been found in the placenta (2 20 26 27 40) In this study the concentrations of $\alpha_1$ antitrypsin was lower and the total antifibrinolytic activity was less in uterine blood than in peripheral blood before placental separation This suggests that the inhibitors present in the placenta seem to be restricted to the fetal circulation where high levels of inhibitors have been found (11 17) The results presented in this study do not support the hypothesis that the inhibitory effect of the placenta on

fibrinolysis depends on its content of fibrinolytic inhibitors.

Åstedt (3) has proposed that the progestagens and estrogens produced by the placenta might be responsible for the depressed fibrinolytic activity during pregnancy and for its return after expulsion of the placenta. The changes in the levels of the placental hormones were not statistically significant in this study. In an extended study of 30 women, in which the patients in this study were a part, the decrease in uterine blood concentrations of both estradiol and progesterone was statistically significant (19). The levels in peripheral blood were unchanged 15 minutes after placental separation.

The possible relationship between the hormonal changes and changes in blood coagulation fibrinolysis was examined but no correlation could be found. Blood was sampled close to the time of delivery in this study and blood sampling two hours after delivery, when the fibrinolytic activity in peripheral blood had returned to non pregnant levels (3, 10, 13, 28, 41), should have given more information. However, a causal relationship can never be proved.

One of the patients studied is of special interest. She had toxemia of pregnancy, a disease in which disturbances in blood coagulation and fibrinolysis are now considered to be involved (12, 23, 24, 38). When this patient is excluded from the statistical analyses the following changes are found: the difference between the plasminogen concentrations in peripheral and uterine blood becomes statistically significant before the placental expulsion and the total antifibrinolytic activity in peripheral blood becomes significantly higher at 15 minutes after placental separation as well as before. The changes in plasminogen concentration are in line with a fibrinolysis in the peripheral circulation before delivery which would explain the appearance of FDP in peripheral blood during pregnancy complicated by toxemia (12, 21, 23, 24). The significance of changes in antifibrinolytic activity eludes explanation at the present moment.

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*Submitted for publication Oct 24 1973*

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Acta Obstet Gynec Scand 54 57–63 1975

# THE EFFECT OF NORETHINDRONE AND SOME OTHER SYNTHETIC GESTAGENS UPON THE PERIPHERAL PLASMA LEVELS OF PROGESTERONE AND ESTRADIOL DURING EARLY HUMAN PREGNANCY

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*Abstract* Synthetic gestagens were given orally (norethindrone d norgestrel chlormadinone acetate) or intramuscularly (17α hydroxyprogesterone caproate) during 5 early human pregnancies The peripheral plasma levels of progesterone and estradiol were determined before during and after the treatment Norethindrone was most extensively used and in those women who received this gestagen the peripheral plasma levels of norethindrone were estimated by radioimmunoassay No consistent effect upon the levels of progesterone or estradiol was observed In 23 women no vaginal bleeding and no abortion occurred within about a week after the treatment The pregnancies were terminated surgically Histological examination of specimens from these abortions revealed no differences on comparison with 10 non treated controls Two women aborted after the treatment but judging from the hormonal levels these pregnancies were abnormal and would probably have aborted regardless of the treatment

Synthetic gestagens exert a profound influence upon human ovarian function Doses which inhibit ovulation have been widely used for contraceptive purposes Continuous administration of a low dose as used in contraceptive mini pills inhibits ovulation in some women while in others the normal pattern of the peripheral plasma levels of progesterone after ovulation are distorted (9 10) Postovulatory treatment with large doses causes a dramatic reduction of the peripheral plasma levels of progesterone (6) and to a lesser extent of estradiol (11)

The question arises whether the administration of synthetic gestagens during early human pregnancy will suppress the plasma levels of progesterone and estradiol and thereby induce an early abortion As exogenous human chorionic gonadotrophin (HCG) has been demonstrated to counteract the effect of postovulatory treatment during the menstrual cycle (6) this possibility appeared somewhat remote However evidence of a less pronounced effect would still be of importance in relation to the widespread use of these substances in the treatment of threatened abortion

The aim of the present investigation was to study the effect of synthetic gestagens during early human pregnancy upon the peripheral plasma levels of progesterone and estradiol in relation to the possibility of inducing vaginal bleeding or abortion

## MATERIAL AND METHODS

*Volunteers* Twenty four healthy women in early pregnancy aged 17–36 years (mean 8) all applying for legal abortion participated in this study One woman participated twice Sixteen of the women had been pregnant at least once before

*General design of the investigation*

A synthetic gestagen was given orally (NET d-norgestrel or CMA)[1] or to one woman intramuscularly (17α hydroxyprogesterone caproate) in various doses and dose schedules

Peripheral venous blood samples for the assay of progesterone estradiol and NET concentrations were collected before during and after the treatment at daily or sometimes longer intervals From those women who were studied already from the time of ovulation

[1] The following trivial names are used Norethindrone (NET) 17α-ethinyl 19-nortestosterone Norgestrel dl 13β ethyl 17α-ethinyl 17 hydroxygon-4-en 3one Chlormadinone acetate (CMA) 6-chloro-6-dehydro 17α-acetoxy progesterone

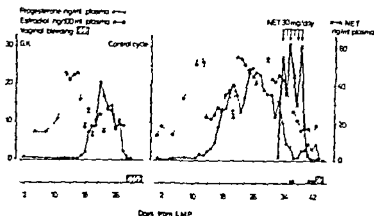



*Fig 1* Case G K Peripheral plasma levels of progesterone estradiol and NET during one normal menstrual cycle and during one early pregnancy from the same woman 30 mg of NET was given orally for 6 days

or shortly thereafter morning samples of urine for the detection of human chorionic gonad trophin (HCG) were collected before or during treatment The de tection of HCG was performed according to Wide (15) using a radioimmunoassay with two different antisera for the discrimination between luteinizing hormone (LH) and HCG

If abortion did not occur within about a week after the end of the treatment cervical dilatation and curettage was performed Fetal tissue was collected for histological examination

Fetal tissue was also collected from 10 non-treated women 8–13 weeks pregnant (mean 11 2 weeks) who were aborted by the same technique

Throughout this study the duration of the pregnan cies was calculated from the start of the last menstrual (L M P)

*rm nal assays*

*esterone in plasma* was determined by a competitive protein binding assay described by Johansson (7)

*Estradiol in plasma* was assayed by a radioimmunoassay according to Hotchkiss et al (4) as described for the assay of human plasma by Edquist & Johansson (3)

*Norethindrone (NET) in plasma* was estimated by a radioimmunoassay described by Nygren et al (13)

## RESULTS

Four women conceived during the collection of blood and urine samples aimed for the determina tion of hormonal levels during the normal menstrual cycle One additional woman suspected pregnancy very early and blood samples were collected from the 32nd day from the L M P The peripheral plasma levels of progesterone estradiol and when relevant NET before during and after the administration of synthetic gestagens to these five women are presented in Figs 1–5

In Fig 1 the plasma levels of progesterone and estradiol during one control cycle are first shown In the following cycle this woman con ceived and HCG was detected in the urine on several occasions before the start of the treat ment with NET which was given orally 30 mg/ day on days 34-39 from the L M P (total 180 mg) During this treatment the plasma levels of NET reached about 60 ng/ml and NET had virtually disappeared from plasma 4 days after the cessa tion of the treatment A small vagin l bleeding occurred on the 36th day and a heavy bleeding on days 41–45 Curettage was not indicated clinically and was therefore not performed The progesterone and estradiol levels mirrored those of the control cycle until about the 22–23rd day At this time the hormonal levels started to in crease When NET treatment was started on day 34 the levels of progesterone had already started to decrease while the levels of estradiol had re mained more constant During treatment the

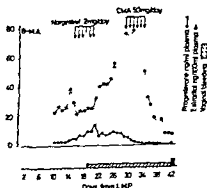



*Fig 2* Case B M A Peripheral plasma levels of progesterone and estradiol from before ovulation in an early human pregnancy during which d-norgestrel and CMA was given orally

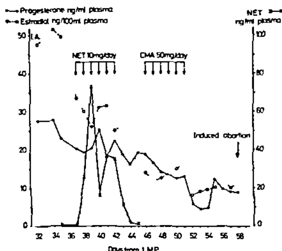


*Fig 3* Case 1 A Peripheral plasma levels of progesterone estradiol and NET in an early human pregnancy during which NET and CMA treatment was given orally

levels of both progesterone and estradiol decreased continuously After treatment a small transient rise of the levels occurred

In the woman represented in Fig 2 oral treatment with d norgestrel was intended to start after ovulation but the treatment was given too early and ovulation is likely to have occurred during the treatment After the norgestrel treatment the levels of estradiol increased continuously progesterone remained elevated and HCG was found in the urine Treatment with CMA 50 mg orally per day for 6 days was initiated on day 29 During this treatment both progesterone and estradiol levels decreased A small vaginal bleeding had started on day 19 and continued until the 42nd day when a heavy bleeding occurred At this time progesterone levels had been very low for more than a week and the levels of estradiol had declined continuously A curettage was performed and upon histological examination a decidual reaction but no chorionic villi were found

During the early pregnancy presented in Fig 3 HCG was found repeatedly in the urine The levels of both progesterone and estradiol had started to decline before treatment with NET which was given orally 10 mg/day for six days starting on day 37 from the L M P During this treatment a peak level of NET of about 35 ng/ml plasma was obtained Progesterone and estradiol continued to decline during and after treatment Eight days after the NET treatment a second period of treatment was started this time with CMA which was given for 6 days During this treatment progesterone continued to decrease slowly while the levels of estradiol showed a transient rise No vaginal bleeding occurred and on the 58th day an abortion was performed surgically Histological examination showed a decidual reaction but no chorionic villi

Fig 4 shows the plasma levels of progesterone and estradiol in a woman in early pregnancy from before ovulation until abortion was induced During the treatment with NET 100 mg orally per day for 13 days progesterone levels decreased somewhat while the levels of estradiol increased continuously The plasma levels of NET rose to about 400 ng/ml No vaginal bleeding occurred and an abortion was performed on the 53rd day Chorionic villi were found microscopically and minor areas of necrosis near chorionic invasive cells were observed

Fig 5 represents a woman to whom 100 mg of NET was given for two days after a rather slow rise of progesterone levels which decreased somewhat after the treatment The levels of estradiol rose continuously before during and after the treatment NET reached a concentration of 200 ng/ml plasma No vaginal bleeding

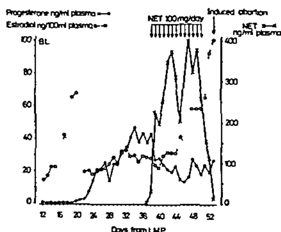


*Fig 4* Case B L Peripheral plasma levels of progesterone estradiol and NET from before ovulation until abortion was induced on day 53 in an early human pregnancy during which NET treatment was given orally

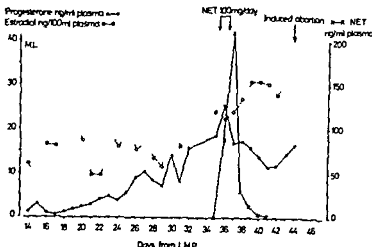



*Fig 5* Case M L Peripheral plasma levels of progesterone estradiol and NET from before ovulation until abortion was induced *in an early human pregnancy* during which NET treatment was given orally

occurred and an abortion was induced Chorionic villi were found histologically

The remaining pregnant women in this study were treated at a somewhat later stage of gestation In these women only one blood sample was taken before the administration of the gestagen

Thirteen women 35–56 days pregnant got different doses of NET during different periods of time Fig 6 presents a composite of the total amount of NET given the mean plasma levels of NET progesterone and estradiol before *during and after the treatment A transient* but statistically significant decrease ($p<0.01$) of the levels of progesterone was found during treatment while the levels of estradiol rose steadily No vaginal bleeding occurred in any of the women and all were aborted surgically In all instances chorionic villi were found and minor necrosis close to chorionic invasive cells was seen

In Fig 7 the levels of progesterone and estradiol in three individual pregnant women treated with *d norgestrel are shown* No definite changes of the levels were seen but it seemed that progesterone levels decreased in one woman No vaginal bleeding occurred Abortion was performed *and chorionic villi were found in specimens* from all three women Necrosis was found near chorial invasive cells

In Fig 8 corresponding findings after treatment with CMA are presented No constant changes of the hormonal levels were found except for a considerable fluctuation of the progesterone levels in one woman No vaginal bleeding occurred The women were aborted surgically

Chorionic villi were found in all women and necrosis was again seen near chorionic invasive cells

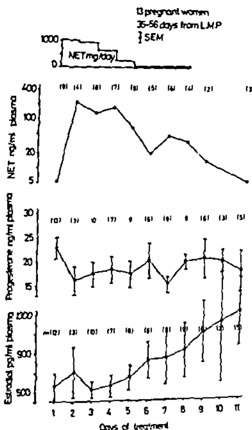



*Fig 6* A composite from 13 early human pregnancies during which NET was given orally indicating the total amount of NET given and the mean peripheral plasma levels of NET progesterone and estradiol The pregnancies were synchronized to the day of the start of the treatment

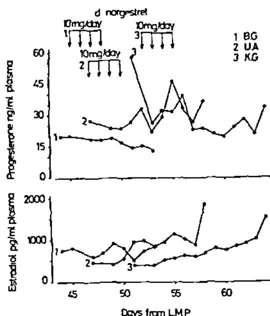



*Fig 7* Peripheral plasma levels of progesterone and estradiol in three early pregnant women treated orally with d norgestrel

One woman received an intramuscular injection of 500 mg 17α hydroxy progesterone caproate on the 61st day of pregnancy No consistent changes of the levels of progesterone or estradiol were found No bleeding occurred and 6 days after the injection an abortion was made Chorionic villi were found and necrosis was found at the same localisation as previously described

Fetal tissue was collected at the legal abortion of 10 women (8–13 weeks pregnant mean 11 2 weeks) who received no hormonal treatment These abortions were performed with the same technique as used for the group of treated women Chorionic villi were found in all specimens and minor necrosis appeared near chorionic invasive cells The histological appearance did not differ between treated and non treated women

## DISCUSSION

The evaluation of the patterns of the peripheral plasma levels of progesterone and estradiol is difficult during the very early stages of pregnancy The normal pattern and range of the levels has not yet been established According to a preliminary report from Tulchinsky 1973 (personal communication) the plasma levels of estradiol rises sharply after the 6th week For progesterone only a relatively limited material has been presented (5) indicating the mean level to be about 25 ng/ml plasma in the 5th week (range 13–38) with a trend of decreasing levels until the 9th week (mean 16 7 ng/ml plasma range 8–31) After the 9th week the levels rise again

Five different synthetic gestagens were given in a wide range of doses and dose schedules

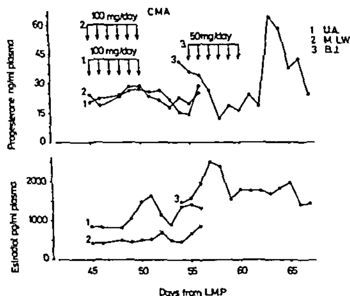



*Fig 8* Peripheral plasma levels of progesterone and estradiol in three early pregnant women treated orally with CMA

The contraceptive potency is considerably higher for NET and d norgestrel as compared to the other gestagens used NET as opposed to d norgestrel and CMA has no pregnancy maintenance capability in the rat (2)

As judged from the hormonal patterns it seems likely that the two abortions (Figs 1 and 2) in this study were not caused by the treatment but would have occurred anyway In one woman (Fig 1) the progesterone levels were already declining when the treatment was started and the other woman who aborted (Fig 2) had progesterone levels higher than 10 ng/ml plasma on only one occasion and vaginal bleeding had occurred two days after ovulation In the pregnancy presented in Fig 3 it seems probable that a spontaneous abortion might have occurred if it had been possible to postpone the abortive operation As no chorionvilli were found an extrauterine pregnancy cannot be totally excluded in any of these three women However there were no clinical signs or symptoms to support such a suspicion The two pregnancies presented in Figs 4 and 5 show a different hormonal pattern as compared with the previous three pregnancies In these two women the treatment might have depressed the levels of progesterone to some extent but the decline might also have been quite normal at this gestational age The levels of estradiol increased continuously It appears therefore that these two pregnancies represented a more normal hormonal pattern as compared with the three pregnancies previously discussed

In the remaining 20 pregnancies which were of a more advanced gestational age than the previous five the administration of NET (Fig 6) d norgestrel (Fig 7) CMA (Fig 8) or 17$\alpha$ hydroxyprogesterone caproate seem to have had minimal effect upon the plasma levels of progesterone and estradiol and no effect upon the early clinical course of gestation The histological appearance of specimens from the aborted women who were treated was not different from that of the 10 untreated women These untreated pregnancies were of somewhat more advanced gestational age but it seems justified to draw the conclusion that the treatment with gestagens did not have any significant effect upon the histological appearance of the product of gestation

It seems reasonable to assume that treatment with synthetic gestagens in early pregnancy exerts a small but at least with NET significant transient effect upon the peripheral plasma levels of progesterone but not upon the levels of estradiol It is possible that the treatment to some extent interferes with the production of steroids from the corpus luteum of pregnancy as it does with the function of the transient corpus luteum during the menstrual cycle (6) However HCG has been shown to counteract the effects upon the corpus luteum during the menstrual cycle (6) and the large amount of HCG present during early pregnancy therefore explains the absence of any drastic effect It is possible that the treatment might have a small effect also upon the production of progesterone from the placenta as it has been recently reported that NET and other 19 nor testosterone derivatives have an inhibitory effect in vitro upon the production of progesterone in the human placenta (8)

However in spite of the administration of different synthetic gestagens in different doses and schedules in the present investigation the effect *in vivo* was found to be very small if any

The plasma levels of NET showed great variations This is probably due to the short plasma half life which has been found for this gestagen (13) and the fact that the blood samples were not collected at the same time following ingestion of NET each day

Oral administration of estrogens to early pregnant women was found by Bačić et al (1) not to affect the clinical course of the pregnancies It seems therefore that once implantation has occurred administration of neither estrogens nor gestagens has any influence upon the maintenance of the pregnancies

Synthetic gestagens as well as progesterone have been widely used in the treatment of threatened abortion but no substantial proof seems to have been presented to support the benefit to such treatment (14) It seems that the vast majority of women with a threatened abortion who subsequently do not abort have normal peripheral plasma levels of progesterone estradiol and HCG (12) According to the findings in the present study the administration of the synthetic gestagens to those women probably does not affect the peripheral levels of progesterone or estradiol However as the levels of progesterone are not consistently depressed the total gestagen

ic load in the peripheral circulation might be increased

The conclusion from this study is that the administration of various doses of various synthetic gestagens during early human pregnancy has no consistent effect upon the peripheral plasma levels of progesterone or estradiol The histological appearance of the fetal tissue was not changed Abortion was not induced by the treatment in normal pregnancies The very marked effect of the same substances during the menstrual cycle is obviously not seen when pregnancy is established

## ACKNOWLEDGEMENTS

This study was supported by the Ford Foundation New York N Y and the Swedish Medical Research Council The authors are indebted to Ass Prof Leif Wide Department of Clinical Chemistry University Hospital Uppsala for kindly performing the discrimination assay between LH and HCG and to Prof Unne Stenram Department of Pathology University Hospital Lund Sweden for expert histological examination of fetal specimens and to the technical staff of the Primate Laboratory for skilled assistance

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*Submitted for publication Nov 16 1973*

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# BOOKS RECEIVED

*Obstetric Therapeutics* Clinical pharmacology and therapeutics in obstetric practice Edited by D F Hawkins 594 pp 55 Figs 2 Plates Bailliere Tindall London 1974 £8 50

For a reasonable price the book gives a good survey of the everyday problems concerning pregnancy fetal growth induction of labour puerperium clinical pharmacology of pregnancy labour and the newborn placental transfer of drugs and finally a short chapter on contraception It is recommended to specialists and post graduate students

*Mental Disorder and Sexuality in the Climacteric* by Tore Hallstrom Reports from the Psychiatric Research Centre St Jorgen s Hospital University of Gothenburg Sweden 6 158 p

Psychiatric thesis with considerable interest also for gynaecologists

*Cytology of the Female Genital Tract* by G Riotton W M Cristopherson International Histological Classification of Tumours No 8 World Health Organization Geneva 27 1973 41 pp illustrated Book which includes accompanying set of 152 transparencies price £19 Sw fr 190 — Book only £6 50 $18 00 Sw fr 65 — French and Spanish editions in preparation

The book gives the result of numerous discussions between the leading cytologists of today The classification reflects the judgement of the members of the International Reference Center It is very well illustrated and should be included into every gynaecological library

*Histological Typing of Ovarian Tumours* by S F Serov and R E Scully World Health Organization Geneva 1973 56 pages Illustrated Price Book which includes accompanying set of 134 colour transparencies £21 $51 00 Sw fr 170 — Book only £6 65 $16 50 Sw fr 55 — French and Spanish editions in preparation

Classification and typing of ovarian tumours belong to the most difficult part of clinical pathology After more than eleven years of work the WHO International Reference Center presents its histological classification of ovarian tumours All illustrations are in colour and of good quality The book is recommended to all departments of obstetrics and gynaecology

*Immunology of Reproduction* Proceedings of the 2nd International Symposium held in Varna Bulgaria 1971 Bulgarian Academy of Science Press 36th Street Sofia 13 Bulgaria 1973 757 pp

Contains 116 papers concerning immunology in human and veterinary reproduction from 26 different countries including the Scandinavian ones

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Acta Obstet Gynec Scand 54 65–70 1975

# THE DIAGNOSTIC VALUE OF RENOGRAPHY IN SUSPECTED OBSTRUCTION OF THE URINARY TRACT DURING PREGNANCY

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*Abstract* The renographic pattern of excretion in 93 pregnant women with clinically suspected obstruction of the urinary tract was not found to differ significantly from what could be expected on the basis of previous reports concerning normal pregnancy It is concluded that in pregnancy the renographic diagnosis of possible pathological urinary tract obstruction is seriously impeded by the very high incidence of impaired excretion of physiological character especially on the right side In cases of pain suggesting urinary tract obstruction the finding of even severely impaired excretion does not conclusively establish an etiological relationship The main importance of renography is to exclude or confirm abnormalities of renal uptake function which may require further investigation or treatment during or after pregnancy Immediately following delivery persisting renographic impairment of excretion suggests an obstruction of pathological significance

Since the introduction of radio isotope renography in 1956 (25) the method has been generally accepted as a simple means of estimating both secretory (uptake) and excretory (drainage) function of the individual kidney (5 7) The safe application of renography during pregnancy has been satisfactorily established with respect to the risk of radiation (16 18 27) and a few reports of the findings in normal pregnancy (2 10 14 17 18 20) as well as in pregnancy complicated by pre-eclampsia (12 17) have been published During normal pregnancy a high incidence of delay in excretion has invariably been reported Occurring as early as the twentieth week of gestation (?) the impairment develops with rising gestational age and disappears immediately following delivery (10 20) The delay is significantly more common and more pronounced on the right side The condition has been found to be influenced by posture with partial relief when the patient changes from supine to a lateral (2 14) sitting (17 18) or genupectoral (10) position

In view of the known influence of renal tract dead space volume on the renographic picture of excretion (3) the reported findings are in accordance with previous reports of dilatation of the urinary tract during normal pregnancy (1 6 8 10 11 15 23 24) Recognized already by Morgagni more than 200 years ago the early observations of dilatation were occasionally made at autopsy and often associated with the cause of death of the patient The development of radiological methods however revealed the frequent existence of dilatation also during uncomplicated pregnancies and in the puerperium and the condition has sometimes been designated as physiological More common in primigravidae (1 10) and sometimes beginning as early as the tenth week of pregnancy (1) the dilatation increases with gestational age It involves both the pyelo-calyceal system and the ureter down to the level of the pelvic brim leaving the intrapelvic part unaffected It is generally agreed that the dilatation is more common and more accentuated in the right urinary tract

Most investigators have adopted the hypothesis that the condition is chiefly due to ureteric obstruction caused by mechanical pressure exerted by the enlarged uterus at the level of the pelvic brim (1 6 10 23) The right sided dominance of the dilatation is explained by anatomical side differences in the position of the pregnant uterus and in the course of the ureters as well as by the

protective effect of the sigmoid on the left ureter. This theory is supported by the observation that renal drainage during pregnancy is influenced by the position of the patient (2, 10, 14, 17, 19). According to another theory the dilatation is primarily due to hormonally induced hypotonia of the urinary tract (9, 26). This theory is contradicted by pressure recordings which reveal a bilateral increase in tonus of the upper urinary tract during pregnancy (21), which finding, however, does not exclude that hormonal factors may have a predisposing effect.

The diagnostic value of renography in cases of clinically suspected obstruction of the urinary tract has not been previously studied. Pointing to the wide range of normal, Nieminen et al. (18) discussed the hypothetical diagnostic limitations of the procedure and suggested special criteria of normal excretion during different stages of pregnancy.

The present investigation deals with the applicability of renography in the diagnosis of clinically *suspected obstruction of the urinary tract during* pregnancy.

## MATERIAL

Ninety three pregnant women were subjected to renography in the course of clinical routine examination occasional or persisting pain in the abdomen or kidney region. In 75 cases the pain was right sided, in 17 cases left sided and in one case bilateral. On the basis of case history and routine physical examination uterine contractions, intrauterine complications as well as biliary or gastro intestinal disorders were considered less likely as the cause of the pain. The quality of the pain was highly variable but in most cases it was severe enough to justify clinical observation for some days. The suspicion of a urinary tract disorder was further supported by tenderness in either kidney region in 27 cases, microscopic haematuria in 19 cases, macroscopic haematuria in 5 cases and positive urinary culture in 14 cases. In 7 cases urinary culture was not performed. Ten patients had initial fever, but all had normal temperature at the time of renography. In 9 cases a renal calculus was passed during pregnancy. Patients with established toxaemia of pregnancy or previously known chronic renal disease were not included in the material.

Fifty seven of the patients were primigravidae. *Another 6 patients* with previous early legal or spontaneous abortions were in this connection treated as primigravidae. In 43 patients renography was performed in the fifteenth to twenty-eighth week of gestation (second trimester). The remaining 50 patients were examined after the twenty eighth week (third trimester). In 33 cases renography was repeated one or more times before term.

Intravenous urography was performed in 19 cases *where renography had* shown significant delay in excretion.

Following delivery, renography was repeated in 29 cases with significant delay in excretion during pregnancy. When repeated before delivery, the latest renography was used for comparison with the post-delivery renography. In 24 cases this was performed within 8 days post partum and in the remaining five cases the interval was 9 to 15 days.

## METHODS

*[$^{131}$I]hippuran renography*

To block thyroid uptake of any free radioactive iodine iodine solution was given orally in a dose equivalent to 200 mg of iodide two hours before the examination. The patients were hydrated with 800 ml of weak tea ingested during 10–30 minutes beginning 30–60 minutes *before the examination*.

Renography was performed with the patient comfortably seated. The kidneys were located by screening with a hand-detector for maximal activity in the kidney regions after injection of 10 µCi [$^{125}$I] hippuran.

*Two parallel 1¾×2 inch crystal scintillation* detectors registered the $^{131}$I activity in the renal tracts separately. Each detector had a conical collimator with an ellipsoid aperture and a length of 9 cm. With the arrangements used, the practical field of detection at the level of the kidney *and determined to the line of* 50 per cent geometric efficiency, was calculated to be 220×155 mm with circularly rounded short sides. In 24 cases a modified collimator was used with a practical field of detection measuring 170×125 mm. 0.3 µCi/kg body weight of [$^{131}$I] hippuran was *injected* into an antecubital vein. The tracer was diluted and given in less than 0.5 ml of fluid and the needle was immediately flushed with saline.

The renograms were recorded with a paper speed of 10 mm/min. Full range was calibrated to 30 000 counts per min. Time constant was 3 sec.

Urinary output during renography was crudely estimated by arranging for the patients to void immediately before and with a recorded interval after the renography. The mean flow per min was calculated.

*Interpretation of the renogram*

The renogram contour may be divided into three parts often called the vascular, the uptake *and the excretion* segments, according to their assumed main functional importance (Fig. 1). The vascular segment is the initial very rapid rise of the curve which mainly corresponds to the increasing background activity in the kidney and adjacent tissues as well as to early parenchymal *uptake* of tracer. The following gradual rise to the peak is due to uptake and accumulation of test substance in the kidney and renal pelvis before it is evacuated from the field of detection. The peak is followed by a descent

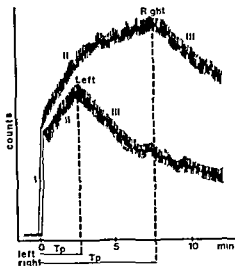



*Fig 1* Renograms in a patient with moderate delay in excretion from the right renal tract Time to the peak *Tp* is indicated for each renogram The roman numerals refer to vascular uptake and excretory segments respectively

of the curve the slope of which chiefly reflects urinary excretion of tracer to the bladder

In the present investigation renal excretory capacity was determined by the time to the peak Tp When renal uptake function is normal Tp has been shown to be linearly related to the quotient between renal tract dead space volume and urinary flow rate (3) Tp was determined as the time elapsing from the beginning of the renogram curve until it reached the maximum value (Fig 1) The time was estimated to the nearest half minute or one-minute interval depending on the sharpness of the peak Sometimes the renogram was still rising when the recording was finished In those cases Tp was taken as exceeding the period of recording which was always longer than 0 min

A Tp of 3 min or less was considered as normal (3) Values of Tp exceeding 3 min were referred to one of the following arbitrary groups 3 5–6 min 6 5–1 min and >1 min which were considered to reflect slight moderate and severe delay of excretion respectively

## RESULTS

The incidence and degree of delay in excretion during pregnancy is shown in Table I In 43 patients in the second trimester of pregnancy a delay of variable magnitude was found in 70 per cent on the right side and in 23 per cent on the left A more than slight delay was established in 33 per cent on the right side and in only 2 per cent (one case) on the left This pattern of impaired excretion was still more pronounced in the third trimester of pregnancy when in 50 patients a delay of excretion was found in 80 per cent on the right side and in 46 per cent on the left The delay was more than slight in 44 per cent on the right side and in 14 per cent on the left

In 33 cases with impaired excretion renography was repeated one or several times during pregnancy in all comprising 60 additional renographies A return of excretion to normal was observed in only two cases in both of which the passage of a urinary calculus took place On the other hand a Tp of 20 min or more indicating a very severe delay in excretion was recorded in 15 cases in two of which before the passage of a calculus

In the 75 cases with right sided pain a more than slight delay of excretion on the same side was found in 31 cases (41 per cent) A more than slight delay on the left side was found in four cases (5 per cent) In three of these cases a similar delay was coincident on the right side leaving a delay predominantly on the left side in only one case

In the 17 cases with left sided pain a more than slight delay on the same side was found in three cases all of which had a similar or more accentuated delay on the right side A more than slight delay entirely on the right side was found in two additional cases

Table I *Distribution of time to the renogram peak in 93 patients with suspected urinary tract obstruction during second and third trimester of pregnancy*

The recorded time to the peak is referred to one of the following groups
≤3 min (normal) 3 5–6 min (slight delay) 6 5–1? min (moderate delay) and >1? min (severe delay) *Tp* time to the peak *n* number of cases % percentage of total number of cases in each trimester

| *Tp* (min) | Second trimester | | | | Third trimester | | | |
|---|---|---|---|---|---|---|---|---|
| | Right | | Left | | Right | | Left | |
| | *n* | % | *n* | % | *n* | % | *n* | % |
| ≤ 3 | 13 | 30 | 33 | 76 7 | 10 | 0 0 | ?7 | 54 0 |
| 3 5– 6 | 16 | 37 [illegible] | 9 | [illegible]0 9 | 18 | 36 0 | 16 | 3 0 |
| 6 5–1 | 8 | 18 6 | 1 | [illegible] 3 | 12 | 4 0 | 5 | 10 0 |
| >1? | 6 | 14 0 | 0 | 0 0 | 10 | 0 0 | [illegible] | 4 0 |
| Total | 43 | | 43 | | 50 | | 50 | |

Table II *Comparison between the distributions of time to the renogram peak before and after delivery in 29 patients with significant delay in excretion before delivery*

The recorded time to the peak is referred to one of the following groups
≤3 min (normal) 3 5–6 min (slight delay) 6 5–12 min (moderate delay) and >12 min (severe delay) *Tp* time to the peak *n* number of cases % percentage of total number of cases before and after delivery respectively

| *Tp* (min) | Before delivery | | | | After delivery | | | |
|---|---|---|---|---|---|---|---|---|
| | Right | | Left | | Right | | Left | |
| | *n* | % | *n* | % | *n* | % | *n* | % |
| ≤ 3 | 0 | 0 0 | 15 | 51 7 | 21 | 72 4 | 22 | 75 9 |
| 3 5–6 | 7 | 24 1 | 8 | 27 6 | 7 | 24 1 | 7 | 24 1 |
| 6 5–12 | 12 | 41 4 | 4 | 13 8 | 1 | 3 4 | 0 | 0 0 |
| >12 | 10 | 34 5 | 2 | 6 9 | 0 | 0 0 | 0 | 0 0 |
| *Total* | 29 | | 29 | | 29 | | 29 | |

In one case with bilateral pain excretion was normal on both sides

In four cases with renal calculus renography preceded the passage of the stone In two of these cases Tp was more than 20 min indicating very severely impaired excretion on the right side Excretion was always normal on the left side After passage of the calculus in 9 cases a more than slight delay was observed in two cases both of which became normal immediately post partum

When renography was repeated after delivery in 29 patients with significant delay in excretion during pregnancy a remarkable return to normal was observed in most cases The incidence and degree of delay of excretion in the last renogram obtained during pregnancy in relation to the renogram obtained post partum is shown in Table II Following delivery a more than slight delay was observed in only one case on the right side

As the renographic picture of excretion is significantly influenced by urinary output this was established in 28 cases with impaired excretion The mode of hydration employed was found to result in a mean urinary output of 4 2 ml/min However a very great variation was observed ranging between 0 5 and 14 6 ml/min In only one case the output was below 1 ml/min

## DISCUSSION

By the use of renography several authors have unanimously confirmed a frequent delay in excretion during pregnancy (2 10 17 18 20) The incidence and degree of the delay has however not been firmly established To some extent this seems to be due to earlier lack of standards of renography as well as of adequately defined parameters of interpretation Lately the time to the renogram peak has been shown to be a suitable parameter for simple interpretation and grading of excretion as expressed by the quotient between renal tract dead space volume and urinary flow rate (3) According to published values of the time to the peak (17 20) a significant delay of excretion in the third trimester of pregnancy may be roughly estimated to occur in 40–70 per cent on the right side and in 20–50 per cent on the left Nieminen et al (18) reported a delay predominantly on the right side in 75 per cent of their cases

The figures given roughly correspond to the incidence of dilatation during late pregnancy which has been reported to be between 60 and 100 per cent in the right renal tract in contrast to the 20 to 60 per cent observed in the left renal tract (1 8 11 15 24) The difference between the two sides was accentuated by the almost invariably greater dilatation on the right side The volume of the dilated upper urinary tract has been estimated to range between 20 and 300 ml (6) The normal renal pelvis holds about 6 ml (2–12 ml) (13)

It appears from the renographic and urographic investigations mentioned that the incidence of delay in excretion in normal pregnancy does not differ significantly from that found in the present series Similarly the pattern of the degree of delay in the present material is in reasonable accord with what could be anticipated from the degrees of dilatation found by Baird (1) in an autopsy material Considering the use of radiation at the examinations now in question it seemed unjustified to extend the present study to comprise another series of normal pregnant controls

The similar pattern of excretion in cases of suspected urinary obstruction as compared with normal pregnant controls indicates that the renographic finding of impaired excretion has a limited diagnostic value In case of pain in the back or abdomen on the right side even severely

impaired excretion on the same side obviously does not establish an etiological relationship With special regard to the diagnosis of acute appendicitis a corresponding opinion was put forward by Schumacher (2?) who warned against diagnostic conclusions on the basis of the finding of a dilated right renal tract at intravenous urography

The diagnosis of a possible pathological obstruction on the right side is seriously impeded by the very high incidence of sometimes even severely impaired excretion of physiological character Special normal values during pregnancy as suggested by Nieminen et al (18) cannot solve this problem of interpretation On the other hand in case of pain of the left side a more than slight delay solely or predominantly on the same side suggests a condition of pathological significance

In the present series it was not possible to establish whether the pain originated in the urinary tract or was related at all to impaired urinary excretion On the basis of the clinical criteria given however such a relation may be suspected in many cases Renal calculus was diagnosed in 9 cases in two of which the renographic follow up indicated a coexisting physiological delay of excretion In another 27 cases re-examined after delivery the rapid return of excretion to normal supported a physiological character of the impairment which possibly reflected physiological dilatation of the renal tract during pregnancy Such a dilatation has been reported to be associated with the complaint of pain in about 15 per cent of the cases (1 22) without relation to the degree of dilatation The pain may be very severe with practically no dilatation of the renal tract (1)

It may be concluded that renography is of limited diagnostic value in cases of pain referable to the urinary tract especially on the right side The main importance of the method in such cases will be to exclude or confirm *severe* abnormalities of excretory function which may demand further investigation or treatment during or after pregnancy The suspicion of such an impairment must be based on conventional clinical criteria mainly by the occurrence of moderate to severe recurrent or persisting pain especially when associated with tenderness in the kidney region or haematuria Renography also serves to reveal possible influence of renal uptake function (7) which may follow in cases of severe obstruction of any cause As long as uptake function is normal a conservative treatment with regularly repeated renography is justified in most cases When necessary the clinical evaluation of the condition may be supplemented by intravenous urography (3)

The rapid return of excretion to normal following delivery as reported by Rudolph and Wax (20) was confirmed in the present study This is in contrast to the findings at intravenous urography where some degree of dilatation has been found to remain for weeks to disappear in about 6 weeks (15) This apparent discrepancy may possibly be explained by the different positions of the patient during the examinations in question In dorsal position a physiological compression of the ureter exerted by the still enlarged uterus may persist to some extent In the upright position this compression is relieved and urinary drainage of the dilated tract may as well be promoted by a hydrostatic pressure gradient The hypothesis is supported by the observation by Brezina (4) at intravenous urography that the dilatation persisting during the week after delivery in most cases subsides when the patient changes from the supine to the upright position The renographic finding of a more than slight delay of excretion obtained after delivery and with the patient seated thus justifies further consideration of possible pathological urinary tract obstruction

## ACKNOWLEDGEMENT

The renographic examinations were performed at the Isotope Laboratory Radiotherapy and Radiation Physics Department Sahlgren Hospital Gothenburg Sweden

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Table 1 *The findings in patients for whom the Beolocator® and the ultrasound examination revealed different results of the presence of the IUCD in the uterus*

| Patient | Beolocator® finding | Ultrasound finding | Roentgenological finding | Clinical evidence |
|---|---|---|---|---|
| 1 | Positive | Negative | IUCD not in pelvis | |
| 2 | Positive | Negative | IUCD not in pelvis | |
| 3 | Negative | Positive | IUCD in pelvis | IUCD extracted from uterus |
| 4 | Negative | Positive | IUCD in pelvis | IUCD extracted from uterus |
| 5 | Negative | Positive | IUCD in pelvis | IUCD extracted from uterus |
| 6 | Negative | Positive | IUCD in pelvis | |
| 7 | Negative | Positive | IUCD in pelvis | |
| 8 | Negative | Positive | IUCD in pelvis | |

& Mastroberardino (3) found B scanning to be fully reliable for this purpose in their series of 55 patients They confirmed their results with hysterosalpingography The ultrasonic method also has the advantage that it detects possible early pregnancy from the sixth week onwards Moreover ultrasonic examination is perfectly harmless for the fetus

Previous investigations did not usually include comparisons of the different methods used for locating the IUCD The purpose of the present work is to compare the Beolocator® method previously in use with the ultrasonic B technique A further purpose is to ascertain the fate of the missing IUCDs

## MATERIAL AND METHODS

The investigation was carried out at the Department of Obstetrics and Gynecology University of Oulu The series consisted of 44 patients admitted to the gynecological out patient department because of the disappearance of the IUCD markers The time period between the insertion of the IUCD and the examination varied from 1 month to about 7 years The types of IUCD included Lippes Loop® Saf T-Coil® Dalcon Shield® and Copper T® However most of the patients did not remember the type of their IUCD Patiens with IUCDs originally without a marker tail were excluded from the study The ages of the patients varied between 21 and 40 years

The examination was generally started with the Beolocator® probe [1] This apparatus has been constructed for detection of foreign bodies in human tissues The metal probe is connected to the equipment by a cable and the contact between the probe and the IUCD can be heard as a characteristic click over the loud speaker However in cases of delayed menstruation no probe examination was made The result of the Beolocator® examination was considered positive if the amplifier produced a sound typical of IUCD contact The examination was then immediately continued with B-scanning The scan was obtained using a Kretztechnik 4100 MG S apparatus with a 2 mHz probe and the full bladder technique Oil was used as a contact medium between the skin and the probe Longitudinal examination was performed in all cases and some patients further underwent a transverse examination The ultrasonic findings were examined for echoes of a foreign body in the uterus Possible signs of early pregnancy in the uterine cavity were also observed The findings were confirmed by AP roentgenography of the pelvis during the following postmenstrum if the finding by one or both of the methods was negative Roentgenological examination was made in 18 cases It was not performed if pregnancy was suspected The finding was confirmed by extraction of the IUCD in 4 cases by legal abortion in 2 cases and by delivery in 7 cases

[1] Beolocator Type 1700 Bang & Olufsen Struer Denmark

## RESULTS

The Beolocator® examination was performed on 40 subjects of whom 22 were interpreted as having the IUCD in the uterine cavity while the remaining 18 patients displayed negative results No Beolocator® examination was performed on the 4 patients with suspected pregnancy

An ultrasound B scan was obtained for all the 44 patients The finding was in disagreement with the Beolocator® finding in 8 cases (Table I) The IUCD was visible in the uterine cavity of 32 subjects (Figs 1 and 2) 2 of whom further displayed changes typical of early pregnancy in the 7th (Fig 3) and 10th weeks At legal abortion the IUCD (Lippes Loop®) was revealed in the uterine cavity No IUCD could be visualized in the uterine cavity of 12 patients (Fig 4) 2 of whom displayed the normal signs of intra-uterine pregnancy in the 8th (Fig 5) and 17th (Fig 6) weeks in the ultrasonic examination Normal delivery was

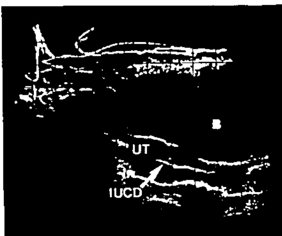



*Fig 1* Longitudinal B-scan Echoes originating from the IUCD (Saf T-Coil®) are visible in the uterus $B$= urinary bladder $UT$=uterus

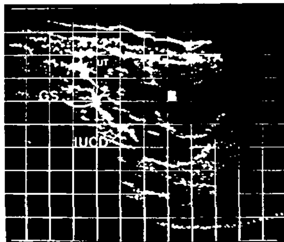



*Fig 3* Gestation sac and IUCD (Lippes Loop®) simutaneously in the uterus in the 7th week of pregnancy $Gs$=gestation sac

later verified in both cases but in one of them the IUCD (Saf T Coil®) was expelled along with the placenta The pregnancy in this case was in the 12th week at the time of the ultrasound examination (Fig 6) and it was not possible to detect echoes of the IUCD among the fetal echoes The negative interpretation of the B scan was thus false The ultrasonic finding was slightly uncertain in one case where the device appeared in the upper part of the cervical canal However the ultrasonic finding was interpreted as positive This device was removed and the positive finding was thus confirmed No clear perforation of the IUCD through the uterine wall was observed

Subsequent roentgenological control was made in 18 non pregnant patients in whom no IUCD could be detected in the uterus by the Beolocator® or ultrasound method The roentgenological findings agreed fully with the ultrasound No IUCD image could be seen in the pelvis if the ultrasonic finding

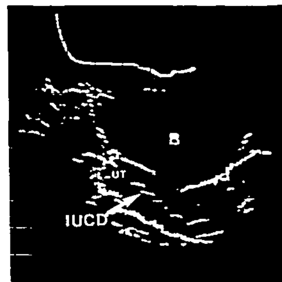



*Fig 2* Longitudinal B-scan Fragmentary echoes of the IUCD (Lippes Loop®) in the uterus

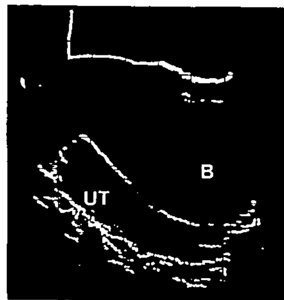



*Fig 4* Normal uterus in the B-scan No IUCD echoes




*Fig 5* Normal intrauterine pregnancy at the 8th week in the B-scan No IUCD echoes *GS*=gestation sac

was negative (10 cases) Comparison of the Beolocator® findings and the roentgenograms showed that the probe method had obviously yielded false negative findings in 6 cases in which an image of the IUCD could be seen in the pelvis despite the negative findings yielded by the probe method Three of these six devices were removed thus confirming their presence in the uterus In all of these 6 cases the ultrasonic finding was positive (Table I) In 2 patients the Beolocator® method gave a false




*Fig 6* Normal intrauterine pregnancy at the 12th week in the B scan Detection of the IUCD (Saf T-Coil®) is not possible among the fetal echoes

positive result No IUCD image could be seen in the pelvis despite the positive *finding by the Beolocator®* method The ultrasonic finding was negative in both cases (Table I)

After the roentgenological and clinical control of the results it is obvious that the explanation for the disappearance of the IUCD markers was expulsion in only 11 cases (25%) and retraction of the threads into the uterine cavity in 33 cases (75%)

## DISCUSSION

In this study two methods for the location of a missing IUCD were examined Besides clinical observations the control method was a plain roentgenogram of the pelvis for the patients in whom one or both of the methods revealed negative results of the intra-uterine location of the IUCD Thus roentgenograms were used for confirmation of the *expulsion of the IUCD* Roentgenological examination was not used if both of the methods revealed an intra-uterine location of the IUCD The positive roentgenological finding only shows the presence of the IUCD in the pelvis but not *necessarily* in the uterus

The results indicated that the ultrasonic B technique is a much more reliable method for locating a missed IUCD than the Beolocator® probe The plain AP roentgenograms showed that all the negative ultrasonic findings in non pregnant patients were correct The only false negative result in the ultrasound IUCD examinations was made in the 12th week of pregnancy In this case the echoes originating from the IUCD could not be distinguished from the fetal echoes in the B scan In my opinion the differentiation is just as difficult even in the later weeks of pregnancy *The examination* for a missing IUCD must therefore be performed before the 11th–12th week if pregnancy has started despite the IUCD Before the 11th week of a *normal pregnancy the gestation* sac can be revealed in the utering cavity by ultrasound B scanning (Fig 5) The echoes of the IUCD can clearly be distinguished from this sac as is shown in Fig 3 However after the 11th week this gestation sac disappears in the B scan and only the various fetal echoes are visible in the uterus

The roentgenological control examination revealed two certain false positive Beolocator® findings and six obviously false negative ones *Rosen* (9) obtained no false findings with this method However false positive findings are possible as

the sound of the probe touching the solid uterine wall may be deceptively similar to the sound of IUCD contact Three of the six false negative findings are certain since the location of the IUCD was confirmed by extraction from the uterus The three other falsenegative findings must be regarded as somewhat uncertain the positive results in the roentgenograms only verified the presence of the IUCD in the pelvic region but not necessarily in the uterus However in all these six cases the ultrasonic finding was clearly positive indicating the intra uterine location of the IUCD

The false negative Beolocator® findings were recorded for patients whose uterus was in very clear ante or retroflexion In such cases it was sometimes difficult to introduce the probe into all parts of the uterine cavity Beolocator® examination further has the disadvantages that it cannot be used when intra-uterine pregnancy is suspected and that it involves the risk of infection

The present results seem to suggest that the disappearence of the IUCD markers is clearly more often due to retraction of the marker threads into the uterine cavity (75%) than to expulsion of the IUCD (25%) This agrees with the findings of Iannıruberto & Mastroberardino (3) Since investigators of expulsion frequency have often regarded the disappearance of markers or a negative probe findings as indicators of expulsion it is possible that the reported expulsion frequencies are in fact higher than is the case in reality

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*Submitted for publication Dec 17 1973*

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# ANNOUNCEMENTS

*An International Conference on Andrology* The Human Semen & Fertility Regulation in the Male will be held on April 24–26 1975 in Gordon Scott Hall Wayne State University School of Medicine Detroit Michigan Sixty speakers and discussants have been invited from Argentina Australia Belgium Canada France Germany Sweden Switzerland United Kingdom and USA Scientific exhibits and films on sperm motility and epididymal physiology are planned A few selected research papers will also be accepted

Those interested in presenting research papers should request forms for Expanded Abstracts Those interested in attending the conference should request Pre registration Forms Write to Program Chairman Dr F S E Hafez Department of Gynecology-Obstetrics Wayne State University School of Medicine 550 E Canfield Detroit Michigan 48201 USA Deadline for applications is Dec 31 1974

*The 21st Symposium of the German Society of Endocrinology* takes place in Munich from February 26 to March 1 1975 Main subjects 1 Established applications of releasing hormones 2 Prolactin—Endocrinology of the Mammary Gland Further information is available from Prof Dr H Karg Institut fur Physiologie 8050 Freising-Weihenstephan West Germany

*The Sixth International Congress of Nephrology* will be held June 8–12 1975 at the Palazzo dei Congressi in Florence Italy For further information please write to the Secretariat Scientific Program Committee Nephrology Dialysis Dept S Orsola University Hospital Via Massarenti 9 40138 Bologna Italy

Acta Obstet Gynec Scand 54 77–84 1975

# STUDIES IN CHOLESTASIS OF PREGNANCY

## *I Clinical Aspects and Liver Function Tests*

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*Abstract* Fifty nine consecutive pregnant women complaining of pruritus without obvious dermatological cause were studied In 57 women the presence in serum of an abnormal lipoprotein LP X characteristic conditions associated with cholestasis was verified by an immunological technique These 57 women were designated as having cholestasis of pregnancy Clinical symptoms were related to liver function tests serum bilirubin alkaline phosphatase SGOT and SGPT Compared with women with uncomplicated pregnancy these patients showed a high frequency ($p<0\ 05$) and long duration ($p<0\ 001$) of emesis food and drug idiosyncrasy ($p<0\ 001$) and gall bladder disease ($p<0\ 05$)

Based on liver function tests two groups of patients with different degrees of severity of cholestasis of pregnancy were differentiated Thirteen cases characterized by serum bilirubin >1 2 mg/100 ml and/or SGOT and SGPT >50 units/l were called hepatosis of pregnancy (HP) while 37 cases with pruritus gravidarum (PG) represented a milder degree of cholestasis

Jaundice which tends to recur in subsequent pregnancies was described by Ahlfeldt (cf 10) in 1883 and was described as a clinical entity in the nineteen fifties by Svanborg et al (23 25) and Thorling (27) Many synonymous terms have been used for this syndrome e g recurrent jaundice of pregnancy idiopathic jaundice of pregnancy hepatosis of pregnancy hepatotoxemia cholestasis of pregnancy and obstetric cholestasis A milder form of this complication of pregnancy is known as pruritus gravdarum

Morphological studies of recurrent jaundice of pregnancy have shown changes similar to those of cholestasis Laboratory and morphological changes usually return to normal in the puerperium (2 9 10 27) The pathogenesis of the cholestasis of pregnancy is still obscure Hormones particularly estrogens have been considered etiologically important by provoking hepatic insufficiency excretory (cf 1)

*Pruritus is the initial and dominating symptom* in cholestasis of pregnancy followed by jaundice and a concomitant increase in alkaline phosphatase and serum transaminases Dependent on criteria used in diagnosis the incidence of cholestasis of pregnancy varies in the literature from 0 02 (7) to 2 4 (16) per cent of all pregnancies (9 10 23 25 27)

Cholestatic conditions are known to cause the following characteristic changes in lipids raised free cholesterol and phospholipids In 1969 Seidel and Alaupovic (18 19) isolated an abnormal serum lipoprotein lipoprotein X (LP X) from patients with obstructive jaundice LP X was found electrophoretically with the $\beta$ lipoproteins having a density of 1 040–1 045 g/ml (*low-density lipoproteins*) LP X *contained* large amounts of free cholesterol and phospholipids In the protein moiety albumin and Apo lipoprotein C (Apo-C usually found in very low density lipoproteins) have been identified (18)

In the present publication consecutive cases of pruritus in pregnancy were investigated and also 57 cases without obvious dermatological causes were further studied for clinical features related to the occurrence of LP X in serum and to liver function tests The modified immunological test for the detection of LP X in serum was found to be a useful diagnostic tool in the diagnosis of cholestasis of pregnancy Furthermore certain limits in

liver function tests serum *bilirubin=1 2 mg/100* ml and SGOT and SGPT=50 Units have been suggested for the differentiation of two degrees of severity of cholestasis of pregnancy *pruritus* gravidarum (PG) and hepatosis of pregnancy (HP)

## MATERIALS AND METHODS

From September 1970 through August 1971 consecutive pregnant women complaining of generalized pruritus were investigated Women with a history of previous hepatitis or showing signs of dermatological disease as well as those receiving drugs (except iron and vitamins prophylactically) were excluded Clinical data in these pregnant women were compared with data in two control series One random control series was composed of 189 women with a history of uncomplicated pregnancy delivered during 1971 in the clinic This control series was studied using the answers from a questionnaire (given to the women at time of delivery) The second control series was a retrospective study of medical records from 220 consecutive pregnancies Women delivered on the first and the fifteenth day of each month were chosen The following data were obtained from the medial records age number of primigravidae number of spontaneous abortions history of food and drug idiosyncrasy perinatal mortality and morbidity blood loss at delivery and blood group A third series of 20 pregnant women at term served as blood donors in the study of the abnormal lipoprotein X in serum

Blood samples were drawn in the morning after 12 hours of fasting during the second and third trimester and on the first and seventh day of the puerperium The following liver function tests were performed *serum bilirubin (normal <1 2 mg/100 ml)* alkaline (normal <8 Buch units) SGOT (normal <17 ts/l) and SGPT (normal <17 Units/l) and ana methods

The semi-quantification of LP X was performed by double immuno-diffusion (15) in 1 per cent agar (Bacto-agar Difco) gels employing barbital buffer pH 8 6 ionic strength 0 05 Rabbit serum containing antibodies to human LP X was obtained through the courtesy of Dr D Seidel Heidelberg West Germany Two different batches of equivalent antibody titre were used All plates were kept in a moist chamber at room temperature The immuno-precipitin lines were visually evaluated after 12 and 36 hours The intensity and the areas of *the immuno-precipitin line closest to the antibody well* were evaluated according to a four-grade scale The evaluation was performed by a colleague unaware of the patient s clinical and laboratory data.

Conventional statistical methods were *used for the* calculation of means standard deviations and standard error of means Student s t test was used to study differences between the means of two groups Linear regressions were calculated according to the method of least squares. Qualitative data were compared by means of chi-square analysis A value of $p<0.05$ was considered to be statistically significant.

## COMMENTS TO METHODS

LP X the abnormal lipoprotein characteristically found in serum in cholestasis shares antigenic *determinants with normal very-low-density-lipoproteins* (VLDL) When non-cholestatic whole human serum is tested by immuno-double-diffusion against LP X anti serum the presence of normal amounts *of VLDL frequently* causes the appearance of an immuno-precipitin line located closer to the antigen well This immuno-precipitin line develops slowly In cholestatic serum in the presence of LP X and VLDL a second *immuno precipitin line* develops closer to the antibody well and appears as a thin line The intensity of the two precipitin lines can be evaluated separately The identities of the VLDL and the LP X immuno-precipitin lines respectively were confirmed by repeated experiments (Fig 1) Reaction of identity was obtained between the *thin immuno-precipitin line closer to* the antibody well and a preparation of purified LP X isolated according to a modification (to be published) of an earlier described *method (18) In the presence of two precipitin lines (that of VLDL* and that of LP X) the elimination of VLDL from whole serum by precipitation was used VLDL was quantitatively precipitated by 0 2 ml 1% Heparin (Vitrum Stockholm Sweden) *and 0 15 ml of* 0 25 M manganese chloride added to *1 1 ml of*

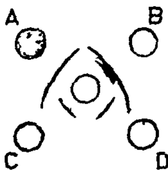



*Fig 1* Immuno-double diffusion patterns of serum *from patient with cholestasis of pregnancy* containing *LP X and very low-density lipoproteins* (B) serum from *same patient after* the elimination of very low-density *lipoproteins by precipitation* (D) a purified sample of *LP X isolated by a modification (to be published)* of an *earlier described method* (Seidel Alaupovic et al ) (C) and *very low-density-lipoproteins* isolated by preparative ultracentrifugation from patient with hypertriglyceridemia (A) *The central well contains anti* LP X serum For explanation see Comments to Methods

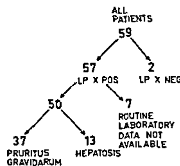



*Fig 2* Outline of study with absolute numbers of pregnant women at each stage of the investigation Clinical data are given in the text for 57 LP X positive pregnant women with pruritus and liver function tests for 50 LP X positive women with complete laboratory data For definition of pruritus gravidarum and hepatosis see Discussion

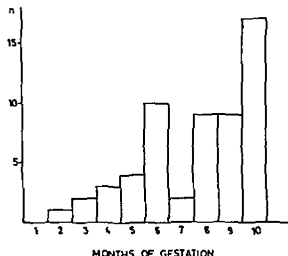



*Fig 3* Debut of pruritus in relation to month of gestation in 57 women with cholestasis of pregnancy Number of cases

serum Precipitated VLDL was removed by low speed centrifugation (8 000 RPM) for 20 minutes The clear phase obtained after the precipitation of VLDL revealed only the precipitin line of LP X

Of 59 *pregnant women with pruritus* without history of previous hepatitis or signs of dermatolo gical disease 57 women revealed the presence of LP X in their sera Only these 57 pregnant women with pruritus have been designated as having cholestasis and are included in the results In 7 women complete laboratory data were missing in the residue of 50 LP X positive cases data on liver function tests are presented (Fig 2)

## RESULTS

*Pruritus* According to definition (Ikonen) pruritus in cholestasis of pregnancy is generalized with a preferential localization in the skin of the extremities including the palms and soles Only one patient complained of itching on her face The onset of pruritus was usually (in 65%) during the third trimester The occurrence of new cases of pruritus due to cholestasis was highest in the sixth and in the eighth ninth gestational months (Fig 3) With reservations for the relatively low number in the present series the two-peak curve for the onset of pruritus is similar to that found by Thorling (1955)

*Emesis* (Table I) Emesis was more common ($p<0\ 05$) among patients with cholestasis (75%) than in the control series (58%) Emesis was of longer duration ($p<0\ 001$) (Fig 4)

*Miscellaneous symptoms* (Table I) Gall bladder disease was more frequently noted ($p<0\ 05$) in pregnant women with pruritus than in the control series Three women earlier operated on for gall bladder disease had experien

Table I *Frequency and duration of emesis occurrence of gall bladder disease and of food and drug idiosyncrasy in cholestasis of pregnancy and in controls*

| | Emesis (*n*) | Duration of emesis (months) | Gall bladder disease (*n*) | Food and drug idiosyncrasy (*n*) |
|---|---|---|---|---|
| Cholestasis of pregnancy | 43/57 (75%) | 3.3 | 9/57 (16%) | 13/57 (23%) |
| Control series | 110/189 (58%) | 1 7 | 13/189 (7%) | 13/220 (6%) |
| *P*< | 0 05 | 0 001 | 0 05 | 0 001 |

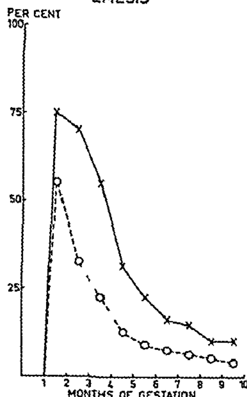



*Fig 4* Duration of emesis in cholestasis of pregnancy (n=57) and in a control series of women with uncomplicated pregnancy ($n$=189) Per cent of total number in each series Cholestasis of pregnancy x x control series o-o

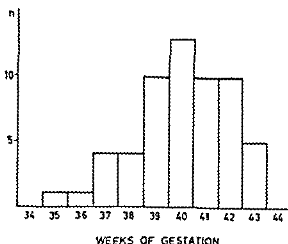



*Fig 5* Delivery in relation to week of gestation in 57 women with cholestasis of pregnancy Number of cases

typical biliary colic The incidence of radiologically proven gall stones appears to be higher 35 % (10) and 30 % (26) respectively

Food and drug idiosyncrasy was more common ($p$<0 001) *in cholestasis of pregnancy* The idiosyncrasy was to penicillins sulfonamides strawberries fish and oranges This appears to be an observation not commented on before in cholestasis of pregnancy

*Obstetric data* In the series of cholestasis of pregnancy and in the controls (at the time of delivery) mean age (27 3 and 26 9 years respectively) median age (26 years) and age distribution did not differ

The time of delivery (Fig 5) showed a normal distribution in patients with cholestasis of pregnancy with a median through the 40th gestational week This is apparently in disagreement with the observations by Thorling (1955) who found a mean gestational age at term in jaundice in a late pregnancy to be 37 weeks The frequency of spontaneous abortions was similar to that found in *the control series as was the proportion of primiparas* (7) Uterine bleeding during delivery was

Table II *Obstetric data Frequency of spontaneous abortion primiparas bleeding at delivery and blood group distribution in cholestasis of pregnancy and in controls*

| | Spontaneous abortion ($n$) | Primiparas ($n$) | Bleeding at delivery[a] (ml) | Blood group distribution[b] A (%) | B | AB | O | Rh + | Rh − |
|---|---|---|---|---|---|---|---|---|---|
| Cholestasis of pregnancy | 11/57 (19 %) | 30/57 (53 %) | 205±24 | 51 | 5 | 5 | 39 | 85 | 15 |
| Control series | 32/220 (15 %) | 105/220 (48 %) | – | 47 | 11 | 5 | 37 | 85 | 15 |
| $P$< | N S | N S | – | | | – | | | – |

[a] Mean±S E M
[b] Data in control series from B Gullbring (8)

Table III *Pediatric data Perinatal mortality dys and immaturity and weight at birth of infants of mothers with cholestasis of pregnancy and of infants of mothers in control series*

| | Perinatal mortality (n) | Immaturity (n) | Dysmaturity (n) | Birth weights* (g) |
|---|---|---|---|---|
| Cholestasis of pregnancy | 1/57 | 1/57 | 4/57 | 3 513±66 |
| Control series | ?/189 | 4/189 | 10/189 | 3 463±40 |
| *P*< | – | – | N S | N S |

Mean±S E M

well within the limits of that of normal pregnancy Seven per cent (4/57) of the women with cholestasis had uterine bleeding exceeding 500 ml The same frequency of major uterine bleeding was found by Friedlander (1967) This excludes a more general defect in coagulation due to cholestasis The distribution among A B O and Rh blood groups was within the expected range (8) (Table II)

*Pediatric aspects* (Table III) The new born infant of the mother who had suffered from pruritus during pregnancy was not afflicted with a higher perinatal mortality nor was the incidence of immaturity and dysmaturity higher than in the control series These results are in agreement with data by Thorling (1955) and Svanborg & Olsson (1959) but in disagreement with other data (7 9 10) where a high perinatal mortality (25 per cent) due to various causes have been described

*Liver function tests* (Fig 6) In the present series of pregnant women with pruritus due to cholestasis the liver function tests serum bilirubin alkaline phosphatase SGOT and SGPT showed a wide range of variation In only three cases was serum bilirubin elevated (>1 2 mg/100 ml) while only a minor portion of the values (9/50) of alkaline phosphatase were within the normal limits (>8 Buch units) given for non pregnant women The levels of alkaline phosphatase increased with consecutive weeks of gestation It should be remembered however that during pregnancy alkaline phosphatase values are expected to increase (by approximately three times) due to heat stable phosphatases produced by the placenta (14) The values of SGOT and SGPT followed a similar pattern to that of alkaline phosphatase in relation to the period of gestation Forty-eight and 44% of the analyses ($n$=50) fell above the upper normal limit (<17 Units) for SGOT and SGPT respectively Normal SGOT and SGPT values are the usual finding (5 10 12) in uncomplicated pregnancy Elevated values of alkaline phosphatase SGOT and SGPT showed a rapid decrease during the first week of the puerperium (Table IV)

In 59 pregnant women with pruritus without a previous history of hepatitis or signs of dermatological disease the cholestatic cause of the pruritus

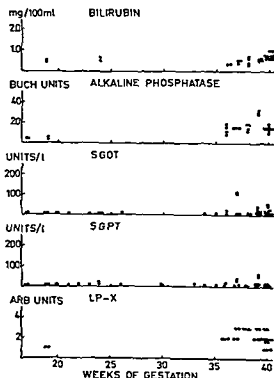



*Fig* 6 Initially obtained values of liver function tests serum bilirubin alkaline phosphatase SGOT SGPT and LP X in 50 women with [illegible] of pregnancy

Table IV *Liver function tests serum bilirubin alkaline phosphatase SGOT SGPT and LP X in cholestasis of pregnancy before and after delivery*
Mean±S E M

| | Last month of pregnancy ($n$=27) | Day 1 of puerperium ($n$=31) | Day 7 of puerperium ($n$=31) |
|---|---|---|---|
| Serum bilirubin (mg/100 ml) | 0 7±0 1 | 0 7±0 1 | 0 5±0 1 |
| Alkaline phosphatase (Buch units/l) | 17±1 2 | 15±0 9 | 13±0 8 |
| SGOT (Units/l) | 35±6 8 | 31±5 3 | 18±1 5 |
| SGPT (Units/l) | 37±9 4 | 35±8 3 | 22±3 5 |
| LP X (Arbitrary units) | 2 3±0 2 | 1 0±0 2 | 0 1±0 1 |

could be verified in 57 by the presence of LP X *in serum as determined by the immunological* technique (Fig 2) LP X was frequently present in serum together with normal values of alkaline phosphatase SGOT and SGPT and was particularly noticeable in those cases in whom the onset of the pruritus was in the second trimester (Fig 6) The semi-quantificated LP X values were related to the concomitant values for SGOT before delivery ($r$=0 53 $p$<0 01) and on day 7 of the puerperium ($r$=0 56 $p$<0 01) This correlation was not present on the first day of the puerperium possibly due to the influence of SGOT originating from muscle tissue released during labour

*Normal pregnancy is not* associated with the presence of LP X in serum This was confirmed in a control series of 20 pregnant women at term

## DISCUSSION

Women with pruritus during pregnancy have been studied Cases of pruritus with obvious dermatological causes were excluded In the remaining 59 cases a large number (n=57) possesed the abnormal LP X *in serum* The two LP X negative cases could be either false negative (laboratory error) or their pruritus was due to other disease *The* 57 cases of pruritus were designated as having cholestasis of pregnancy

LP X appeared in serum with the onset *of* pruritus and was confirmed as early as the third month of gestation frequently in the absence of other abnormal liver function tests LP X is an abnormal lipoprotein characteristically found in serum in conditions associated with cholestasis and also in the rare inborn error of lecithin *cholesterol acyltransferase* (LCAT) *deficiency* Whether the occurrence of LP X in serum is due to an influence of bile acids on lipid/protein synthesis in the liver or is due to changes in the intravascular metabolism of serum lipoproteins is still not confirmed The presence of the abnormal lipoprotein X (LP X) in serum has not been used before as a diagnostic tool in cholestasis of pregnancy

Cholestasis of pregnancy occurs with *different* degrees of severity Utilizing the present data on liver *function tests* an attempt was made to differentiate the more advanced cases of cholestasis with hepatosis of pregnancy (HP) from a group *of milder* cases designated as pruritus gravidarum (PG) In this series of cholestasis of pregnancy 13 cases with more marked *abnormalities* in liver function tests could be separated from 37 milder cases The subgroup of HP was characterized by either abnormally *high serum* bilirubin (>1 2 mg/100 ml) (present in three cases) or elevated SGOT and/or SGPT (>50 Units) (present in 10 and 13 cases *respectively*) The chosen borderline in SGOT and SGPT (>50 Units) would limit the *possibility* of including other than *definite* elevations in these variables into the subgroup of HP

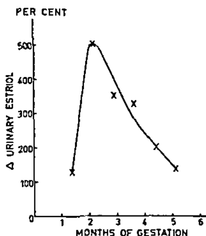



*Fig 7* Relative increase in urinary excretion of estriol during first trimester (calculated from data by Brown 1956)

By this means the diagnosis of HP would indicate a more advanced liver damage although not necessarily associated with jaundice The terms of PG and HP for two different degrees of severity of cholestasis of pregnancy will be used in future publications on this subject

Although the onset of pruritus in cholestasis of pregnancy most frequently occurs during the last trimester the finding in the present series of a prolonged and severe emesis early in pregnancy suggest that the basic and possibly metabolic disturbance causing the cholestasis is present throughout pregnancy Emesis of normal pregnancy has been linked with increased trophoblast activity and HCG excretion It is however also clear that emesis occurs concomitantly with the increase in estrogen activity When the fractional increase in estriol is calculated (from data by Brown 1956) month by month a curve is obtained (Fig 7) with a striking similarity to the emesis incidence curve in cholestasis of pregnancy Some earlier evidence (11 12) support the suggested relationship between estrogen activity and emesis Ethinylestradiol was given during the puerperium to women with a past history of pregnancy with pruritus A similar incidence (70%) of emesis was found as in the present series of cholestasis of pregnancy

The specific cause of pruritus in cholestasis of pregnancy is possibly the presence of increased amounts of bile acids However pruritus is not correlated with the bile acid content of serum but rather with its content in the skin (17) In relation to pruritus in cholestasis of pregnancy attention has also been drawn to the findings of an increased amount of plasma steroid metabolites (particularly pregnandiol) which are said to be partially metabolized by the skin (20 21) In the present study pruritus to some extent followed the variations in LP X

The cause for intra hepatic cholestasis in this condition in pregnancy has not been disclosed Most evidence (cf 1 22) points to the estrogenic components as a likely cause for the cholestasis Pruritus and emesis can be produced by the administration of contraceptive drugs containing estrogenic and gestagenic components (cf 1 11 12) but have not been observed following the administration of contraceptives containing only low dose progestagens (13) On the other hand it has not been possible to verify increased urinary levels of estrogenic components in recurrent jaundice in pregnancy (3) Additional data to be published further support the hypothesis of an increased influence of estrogen or estrogen acting metabolites on liver metabolism

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*Submitted for publication Oct 17 1973*

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Acta Obstet Gynec Scand 54 85–88 1975

# THE URINE–PLASMA RATIO OF SOME PROTEINS IN GESTOSIS

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*Abstract* The urine and plasma content of certain proteins was studied near term in healthy gravidas in gravidas with essential hypertension proteinuria hypertension of pregnancy and gestosis (pre-eclampsia toxaemia) besides this the ratio of urine–plasma concentrations for each protein was calculated In gestosis only the urine–plasma ratio of ceruloplasmin differed from those of the other proteins but in the other diseases studied this ratio showed no difference from that in healthy gravidas The amounts of different proteins excreted in urine did not correlate with the molecular size of the respective protein

A number of studies have shown that gestosis (pre eclampsia toxaemia) is always accompanied by thickening of the glomerular basement membrane and swelling of the endothelium (8 9) which is a result of disseminated intravascular coagulation (4 11) The proteinuria in gestosis is the result of the increased glomerular permeability present in this disease (2) It has been shown that the clearance rate of plasma proteins is dependent upon molecular weight and that it decreases in proportion with higher molecular weight (3 5) This simple selectivity is also found in gestosis (10) Attempts have been made to find for some proteins a urine–plasma ratio characteristic of gestosis (6 12) but no definitely specific protein has been detected Since the discovery of such a specific protein might be of great importance in differential diagnosis and possibly also in making an early diagnosis the present study was undertaken to study some relatively small molecular proteins not only in gestosis but also in certain other related diseases during pregnancy

## MATERIAL AND METHOD

The series studied consisted of 15 healthy pregnant women and [illegible] gravidas with essential hypertension 3 with proteinuria of late pregnancy 17 with hypertension of late pregnancy and 8 with gestosis The criteria of gestosis were a pathological blood pressure and proteinuria. The upper limit of normal blood pressure was set at 140/90 mmHg and the highest normal proteinuria at 0 3 g/24 h measured by Esbach's method Gestosis was considered as a complication of late pregnancy if it had begun in the 26th week of gestation at the earliest At the time of study the pregnancies were all in the 36th–40th week of gestation All the fetuses were alive

Of the collected 24-hour urine 400 ml was concentrated to 10 ml Concentration was done under nitrogen pressure using a magnetic mixer and ultrafiltration apparatus (Amicon N V Oosterhout Holland) The same manufacturer s filter paper XM 50 was used allowing particles of molecular weight 50 000 to remain in the solution to be studied

Twenty ml of blood was obtained from the cubital vein and centrifuged immediately The plasma was studied without concentration

From both groups of samples 8 proteins were determined in mg per 100 ml using radial immunodiffusion containing specific antiserum (Partigen plates Behringwerke AG Marburg BRD) (7) The standard sera of the same manufacturer were used as standards The standard deviation of the method was less than 2% of the mean

The proteins to be studied were selected so as to be representative of different molecular sizes but nevertheless were within the limits of small-molecular proteins since we expected to find within this range a boundary line of glomerular filtration serviceable in this connection Exceptions were Ig M which was chosen to represent a very large size of molecule and Ig-G and ceruloplasmin which were included because of their identical

[1] The immunodiffusion plates were kindly supplied by Behringwerke AG

Table I *Mean absolute amounts of proteins in mg per 100 ml in the different disease groups*

Values differing significantly ($p<0\ 01$) from the values of healthy gravidas are printed in bold face Number of patients stated in parentheses

| | Healthy gravidas (15) | | Essential hyper tension (22) | | Proteinuria (3) | | Hypertension of late pregnancy (17) | | Gestosis (8) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Urine | Plasma | Urine | Plasma | Urine | Plasma | Urine | Plasma | Urine | Plasma |
| Gc globulin | 0 2 | 48 5 | 0 5 | 48 7 | 1.5 | 53 7 | 0 7 | 54 0 | 12 3 | 51 9 |
| Prealbumin | 0 2 | 22 2 | 0 3 | 22 7 | 0 6 | .0 0 | 0 2 | ?1 4 | 1 4 | 24 9 |
| Albumin | 7 5 | 3049 3 | 10 5 | 2869 1 | 138 0 | 2356 7 | 2? 1 | 2674 7 | 117 9 | 2750 6 |
| Transferrin | 0 5 | 361 9 | 1.3 | 374 2 | 93 9 | 311 7 | 9 2 | 39? 8 | 32 6 | 415 9 |
| Haptoglobin | 0 5 | 105 5 | 0 9 | 134 5 | 2 3 | 150 0 | 2 0 | 150 4 | 2 5 | 98 6 |
| Ig-G | 3 0 | 1046 5 | 3 1 | 1011 4 | 35 3 | 448 0 | 7 3 | 916 6 | 30 3 | 914 0 |
| Ceruloplasmin | 0 2 | 33 3 | 0 3 | 43 3 | 3 8 | 53 0 | 1 5 | 54 5 | 7 6 | 27 6 |
| Ig M | 0 03 | 179 5 | 0 2 | 186 0 | 0 7 | 298 0 | 0 5 | 177 5 | 2 9 | 187 7 |

molecular size to demonstrate whether the size of mole cule was a decisive factor The proteins studied were gc globulin prealbumin albumin transferrin haptoglobin immunoglobulin-G (Ig-G) ceruloplasmin and immunoglobulin M (*Ig M*)

To reveal possible selectivity the clearance of each protein in relation to transferrin was calculated from *the formula*

$$\frac{Ux/Px}{Utr/Ptr} \times 100$$

where Ux=urine concentration of the respective protein and Px=plasma concentration Tr=Transferrin (1 13)

Student s *t* test was used for statistical analysis of the results

## RESULTS

The mean absolute amounts of the proteins studied in the various groups are presented in Table I The proteins are listed in order of molecular size Values differing significantly ($p<0\ 01$) from those of healthy subjects are printed in bold face

Only in rare cases did the plasma values differ significantly *from those of the healthy gravidas* Such a difference was not found in any of the pa tients with gestosis On the other hand all the urine protein values with the exception o gc-globulin differed significantly in gestosis from he respec tive values of healthy gravidas In proteinuria the values were very close to those in gestosis

Table II presents the urine plasma ratio of each protein multiplied by 1000 In also this table the value is in bold if it differs significantly ($p<0\ 01$) from that of the healthy gravidas In gestosis the differences are *highly significant* ($p<0\ 001$) for albumin transferrin Ig G ceruloplasmin and Ig M and significant ($p<0\ 01$) for prealbumin The differences were not significant for gc globulin and haptoglobin In proteinuria there were highly

Table II *Urine–plasma ratio of proteins in the different disease groups*

Values differing significantly ($p<0\ 01$) from the values of healthy gravidas are printed in bold face Number of patients stated in parentheses

| | Molecular *size* | Healthy gravidas (15) | Essential hypertension (22) | Proteinuria (3) | Hypertension of late pregnancy (17) | *Gestosis* (8) |
|---|---|---|---|---|---|---|
| Gc globulin | 50 000 | 3 9 | 9 9 | 26 4 | 12 2 | 220 0 |
| Prealbumin | 61 000 | 8 4 | 12 0 | 28 4 | 9 9 | 58 2 |
| Albumin | 69 000 | 2 4 | 3 7 | 58 3 | 8 6 | 45 6 |
| Transferrin | 90 000 | 1 3 | 3.5 | 300 5 | 16 2 | 92 5 |
| Haptoglobin | 100 000 | 6 7 | 7 8 | 15 5 | 10 2 | 4? 6 |
| Ig-G | 160 000 | 2 9 | 3 2 | 85.5 | 9 5 | 37 1 |
| Ceruloplasmin | 160 000 | 5 3 | 6 4 | 79 5 | 38 0 | 356 7 |
| Ig M | 1 000 000 | 0 1 | 1 0 | 2 4 | 5 7 | 17 4 |

Table III *Protein differential clearance (percentage of transferrin clearance)*

| | Healthy gravidas | Essential hypertension | Proteinuria | Hypertension of late pregnancy | Gestosis |
|---|---|---|---|---|---|
| Gc globulin | 290 | 289 | 9 | 75 | 24 |
| Prealbumin | 6 6 | 346 | 9 | 60 | 63 |
| Albumin | 179 | 107 | 19 | 52 | 50 |
| Transferrin | 100 | 100 | 100 | 100 | 100 |
| Haptoglobin | 500 | 274 | 5 | 63 | 52 |
| Ig-G | 214 | 93 | 28 | 57 | 40 |
| Ceruloplasmin | 398 | 183 | 26 | 235 | 387 |
| Ig M | 10 | 29 | 8 | 35 | 18 |

significant differences in the ratios for albumin transferrin Ig G and Ig M and a significant difference in that for gc-globulin The differences were not significant in the case of prealbumin haptoglobin and ceruloplasmin In essential hypertension the transferrin ratio differed significantly from that in healthy gravidas

In Table III are shown the protein differential clearances in the disease groups studied This index values are clearly lower in the diseases complicating pregnancy than in the healthy subjects but there is no uniform finding related to the size of the protein molecule Transferrin and ceruloplasmin form notable exceptions with very high values in the gestosis and hypertension of pregnancy groups Ceruloplasmin has a low index in the proteinuria group in which the transferrin clearance value is higher than that for any other protein

## DISCUSSION

The values obtained are approximately of the same order of magnitude as those presented in the literature (6 12) The values reported by Simanowitz differed markedly from those now obtained but the method used by him for protein determinations was a different one (10)

The maternal plasma protein concentrations do not differ significantly from each other in the various groups studied The present investigation did not reveal in gestosis the increase in large molecular proteins in maternal plasma which has been observed in gestosis and the nephrotic syndrome (13) The proteins now studied were however of relatively small molecular size Patients with proteinuria on the other hand have a significantly higher plasma Ig M concentration than normal cases

As could be expected the urine–plasma ratio of proteins showed relatively little difference in gestosis and in proteinuria Only prealbumin and ceruloplasmin differed significantly from the control in gestosis but not in proteinuria On the other hand marked differences from the values in gestosis were seen both in essential hypertension and in hypertension manifesting during pregnancy

The expected decrease in the urine–plasma ratio of proteins in proportion with higher molecular weight was not seen in either normal or complicated cases of pregnancy Some slight trend may possibly be observable but it is indefinite

If the transfer of a protein through the glomerular capillary wall would be related only to its molecular weight one should expect that Ig-G and ceruloplasmin are excreted in the urine to the same extent The urine–plasma ratio of ceruloplasmin however was found to be remarkably higher than that of Ig-G although their molecular weights are almost the same (ceruloplasmin 148 000–160 000 Ig-G 156 000–161 000) This agrees with observations made earlier in patients with severe proteinuria in non pregnant subjects (14) On the base of the results of the present study the hypothesis was drawn that the rate of clearance of ceruloplasmin and Ig-G by the kidney is influenced not only by molecular weight but also by the shape of the molecule Hence there is no specific pattern for gestosis that could serve as base for differential diagnosis Nevertheless among all the proteins studied the difference in the ceruloplasmin ratio in gestosis and in healthy pregnancy was definitely the greatest in statistical significance Furthermore a similar difference from the normal values for ceruloplasmin was not seen in any of the other disease groups studied

In the differential protein clearance the cerulo-

plasmin clearance was exactly the same in healthy gravidas and in pregnancy complicated by gestosis. That of other proteins was without exception decreased. Although ceruloplasmin and transferrin thus differ greatly in molecular weight, the change in their urine–plasma ratios in gestosis is of equal magnitude. Also this observation does not support selectivity associated with molecular weight. In the other diseases, on the contrary, the mutual relationship between these two proteins differs markedly.

Of the proteins studied, ceruloplasmin appears to be the most sensitive one in revealing changes in the protein levels typical for gestosis. The usefulness of its determination in an early stage of gestosis or prior to manifestation of clinical symptoms may well warrant further investigation.

## ACKNOWLEDGEMENT

This study is supported by grants from the Foundation for Gynaecological Research in Finland.

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*Submitted for publication Jan. 15, 1974*

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Acta Obstet Gynec Scand 54 89–90 1975

# AGING AND URINARY OESTROGEN EXCRETION IN THE MALE

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Conflicting results have been presented about the influence of aging upon the urinary excretion and the blood levels of oestrogens in the male While Behrsohn & Oelefse (2) found an increased urinary excretion of conjugated oestrone oestradiol 17$\beta$ and oestriol with increasing age Pincus (17 18) found a slight continuous decrease in the excretion of oestrone + oestradiol 17$\beta$ and an almost constant excretion of oestriol Kaufmann (13) noted no age-dependent change in the excretion of oestrone and oestradiol 17$\beta$ The studies on blood hitherto reported indicate increased levels of unconjugated oestrone and oestradiol 17$\beta$ with increasing age (8 16 20)

We have studied the influence of aging upon the urinary excretion of low polar oestrogens (oestrone + oestradiol 17$\beta$) in normal healthy males aged 20–79 years The subjects below 50 years of age had proven fertility or normal spermiograms The older subjects had been hospitalized for minor operations There were no indications for endocrinological perturbations or abnormalities in the prostate Values for haemoglobin Na $K^+$ sedimentation rate urinary residual nitrogen and urinary sediment were normal and all patients were free from medications 24 hour urine samples were collected preoperatively Low polar oestrogens were determined according to Carlstrom and Furuhjelm (6)

## RESULTS AND DISCUSSION

The results are given in Table I There was a significant ($p$=0 001) abrupt decrease in the excretion at about 60 years from 5 4 $\mu$g/24 hours (50–59 years) to 3 3 $\mu$g/24 hours (60–69 years)

Approximately 80% of the conjugated low polar oestrogens in male urine consists of oestrone (2 3 9 12 15 19) The sulphoconjugate of oestrone is indeed the most abundant low polar oestrogen in peripheral plasma from males as well as from pregnant and non pregnant females (1 4 7 11 14) No simultaneous assays of conjugated oestrogens in male urine and plasma seem to have been carried out but it is known from studies on pregnant women that the urinary excretion reflects the plasma levels quite well (10) The drop in the low polar oestrogen excretion at about 60 years might therefore reflect a sudden decrease in plasma oestrone sulphate

Vihko has shown that the plasma levels of several neutral steroid sulphates decrease abruptly at about 60 years in males (22) It has been shown by Vermeulen and coworkers (21) that the transformation of injected radioactive testosterone into sulphoconjugated metabolites decreased drastically in older males while the transformation into glucosiduronates remained constant and independent of age The decrease in urine and plasma values for the steroid sulphates might therefore be attributed to a decrease in the sulphurylating activity in the liver It has recently been shown by Carlstedt Duke and Gustafsson (5) that *in vivo* administration of androgens to rats decreased the *in vitro* steroid sulphurylating activity in the liver while oestrogens increased this activity It is therefore tempting to speculate over a regulatory effect of sex steroids on the sulphurylating activity in man Thus it might be possible that an age-dependent drop in the oestrogen production causes a decrease in the liver sulphurylating activity

A decreased sulphurylation of oestrone might lead to an increase in the plasma levels of un

Table I *Influence of age upon the urinary excretion of low polar oestrogens (oestrone + oestradiol 17β) in males*

| Age (y) | n | Low polar oestrogens µg/24 hours | |
|---|---|---|---|
| | | Mean | Range |
| 20–29 | 20 | 5 2±0 4 | 3–8 |
| 30–39 | 16 | 5 0±0 4 | 3–7 |
| 40–49 | 5 | 4 6±0 3 | 2–7 |
| 50–59 | 24 | 5 4±0 5 | 2–11 |
| 60–69 | 26 | 3 3±0 3 | 1–7 |
| 70–79 | 13 | 3 1±0 4 | 1–6 |

conjugated oestrone and oestradiol 17β and this might explain the increased levels of unconjugated plasma oestrogens found in older males (8 16 20) Thus the estimation of unconjugated oestrogens in plasma might not give a true picture of the total oestrogen production in the male We are therefore repeating this investigation using radioimmunoassay of oestrone sulphate in plasma and the results are to be published later

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Acta Obstet Gynec Scand 54 91–94 1975

CASE REPORT

# CERVICAL PREGNANCY

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*Abstract* Within the last ten years 2 cases of cervical pregnancy have been recorded in the Gynecological department of the Odense University Hospital The case histories are given In one of the cases the diagnosis was made clinically In the other the diagnosis isthmico-cervical pregnancy was confirmed by pathological examination The diagnostic criteria and treatment are described and discussed The occurrence of cervical pregnancy is presumed to be more common than assumed to date inasmuch as a number of early cases without complications are probably recorded as spontaneous abortions

The term cervical pregnancy is used for a condition in which nidation and development of the fertilized ovum occurs in the cervix of the uterus Owing to the slight decidual reaction in the mucosa of the cervix the trophoblast will break through the mucosa and infiltrate the underlying tissue Schneider (7) has defined cervical pregnancy as Distal ectopic pregnancy and considered it functionally as a form of extrauterine pregnancy

Cervical pregnancy has previously been considered as an extremely rare condition In 1961 Thomsen & Johansen (10) found approximately 80 reported and 10–20 known but unpublished cases In 1968 Matracaru (4) stated that 182 cases had been reported In Denmark only three cases of cervical pregnancy have been published previously (3 10) of which one (3) ended with the birth of a living child in the 30th week of pregnancy

Within the last 10 years 2 cases considered as cervical pregnancy have been treated in the Gynecological Department of the Odense University Hospital Both case histories are given here with the object of discussing the problems of diagnosis and treatment

## CASE HISTORIES

I A 2? year old gravida 1 with regular menstruation since the age of 14 years Last menstruation 29th October 1962 During the 14th week of pregnancy the patient had a transitory episode of hemorrhage accompanied by slight pain The patient was admitted to hospital during the 18th week of pregnancy owing to bleeding

At gynecological examination it was seen that the cervix was large and distended with a slightly dilatated external os The cervix was the size of a large goose egg tense and painful The uterine body seemed to be of normal size and slightly soft in consistency on bimanual examination Under general anesthesia the thin and effaced external os was dilatated digitally and an approximately 4 cm long macerated fetus was removed together with abortion tissue which was situated just inside the external os The cavity which was 10 cm long could be felt to be divided from the corpus by a narrow internal os Only decidual like tissue was removed from the 4 cm long uterine cavity There was slight hemorrhage during the operation which stopped on packing The pack could be removed on the following day and the patient discharged a few days later

Histological examination showed placental tissue with numerous chorionic villi both in the cervical canal and in the uterine cavity together with large flakes of decidual tissue Histological examination was thus unable to determine the site of the pregnancy

In 1964 and 1966 the patient carried two normal pregnancies and had normal births In 1972 she was subjected to legal abortion

II A 3? year-old para 2 gravida 5 regular menstruation since the age of 14 years admitted 1963 for primary sterility In the following ? years two spontaneous uncomplicated abortions 1965 and 1967 two normal births following uncomplicated pregnancies

Last menstruation 17th October 1971 Seven weeks later admitted to the department following some days of vaginal bleeding and back-ache Gynecological examination revealed a large and plump cervix and closed os without signs of fresh bleeding The uterus was enlarged to the size of a 5–6 week pregnancy There was no bleeding on admission and the patient was dis

Table I *Influence of age upon the urinary excretion of low polar oestrogens (oestrone + oestradiol 17β) in males*

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| | | Mean | Range |
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conjugated oestrone and oestradiol 17β and this might explain the increased levels of unconjugated plasma oestrogens found in older males (8 16 20) Thus the estimation of unconjugated oestrogens in plasma might not give a true picture of the total oestrogen production in the male We are therefore repeating this investigation using radioimmunoassay of oestrone sulphate in plasma and the results are to be published later

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Acta Obstet Gynec Scand 54 95–100 1975

# TREATMENT OF IMMINENT PREMATURE LABOUR

## *A Comparison Between the Effects of Nylidrin Chloride and Isoxuprine Chloride as well as of Ethanol*

Olli Castren Martti Gummerus and Seppo Saarikoski

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*Abstract* In this study 194 imminent premature deliveries were treated The pregnancies were in the 28th to 36th week Uterine contractions were demonstrable in all patients and amniotic membranes were intact All patients were treated with bedrest Two beta sympathomimetics were used in a double blind study Nylidrin hydrochloride (43 cases) and Isoxuprine hydrochloride (60 cases) A placebo was given to 41 patients and ethyl alcohol to 50 patients Intravenous and intramuscular treatment given in the hospital was continued with oral administration at home and follow up examinations were repeated at short intervals Taking a minimum birth weight of 2500 g as the criteria of successful treatment the success rate in the placebo group was 71% in the Nylidrin hydrochloride group 86% the Isoxuprine hydrochloride group 75% and the alcohol group 70% When premature delivery was postponed 7 days the pregnancy advanced to the 37th week or later in 73 77 6 and 56% in their respective groups The beta sympathomimetics especially the Nylidrin hydrochloride were in every respect more efficient than placebo or alcohol The therapeutic effect of alcohol was no better than that obtained with placebo From the fetal point of view the drugs used in the present study showed no adverse effects

The reliability of the results of studies concerning prevention of imminent premature labour is hampered on the one hand by the lack of sufficient material and on the other by the absence of control series In recent years the agents most often used for such prevention include ethyl alcohol and $\beta$ sympathomimetic drugs A double blind study on the effects of ethanol was not published until quite recently (17) and a corresponding study on $\beta$ sympathomimetics has been performed with Ritodrine chloride (16) The purpose of the present work was to compare an adequate series of patients and using double blind trials the drugs most generally used in imminent premature labour These were ethanol and the $\beta$-sympathomimetic drugs Nylidrin and Isoxuprine

## MATERIAL AND METHODS

This clinical trial was performed at Departments I and II of Obstetrics and Gynecology University Central Hospital of Helsinki and in the Midwifery Institute Hospital In both of these hospitals the patients were treated according to similar therapeutic principles

The patients accepted for this study fulfilled the following requirements active uterine contractions were present verified when necessary by means of tocography the duration of pregnancy was 24 to 36 weeks as calculated from the last menstrual period and the amniotic membranes were intact

The $\beta$-sympathomimetics used (Fig 1) were not known to the participating physicians and were Nylidrin hydrochloride and Isoxuprine hydrochloride (we wish to thank the pharmaceutical manufacturers Tropon Werke A G Koln and Laaketehdas Orion Helsinki for making these products available to us) The ethyl alcohol solution for infusion was prepared from 99% pure alcohol The placebo ampoule contained 5 8% glucose and the placebo tablets contained lactose and cornstarch Every other patient arriving at the Department of Obstetrics University Central Hospital of Helsinki who fulfilled the above mentioned criteria was admitted to Obstetric Department I where the product used to suppress premature contractions was K 75/A which subsequently turned out to be Nylidrin chloride and other patients were admitted to Department II where ethanol was used In the Midwifery School Hospital every other patient was admitted to Department I and treated with Isoxuprine and the remaining

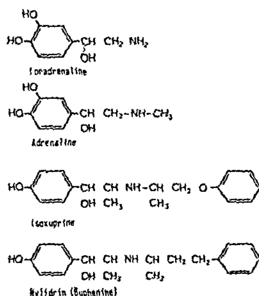



*Fig 1* Structural formulas of Noradrenaline Adrenaline and beta sympathomimetic drugs Isoxuprine and Nylidrin used in this study

patients were admitted to Department II and treated with 75-K/B which turned out to be placebo

Treatment was started immediately on admission *Nylidrin* was administered initially in the form of an intravenous infusion containing 50 mg Nylidrin hydrochloride (10 ampoules) in 500 ml 5% glucose The infusion rate was 0 2 mg/min for one hour then 0 1 mg/min for 5 hours The rate and duration of the infusion could be adjusted if necessary because of the strength of the contractions During the infusion the patients received approximately 50 mg Nylidrin hydrochloride On completion of the infusion Nylidrin was administered i m in a dose of 5 mg (1 ampoule) and thereafter 5 mg i m three times at three hour intervals Thereafter the patients received over 48 hours 5 mg doses of Nylidrin every 5 hours i m and the treatment was then continued by the oral administration of 6 mg (1 tablet) every 5 hours this dosage was also prescribed for the patients following discharge from the hospital

The patients who received placebo were treated according to the same therapeutic schedule

The *Isoxuprine* infusion solution contained 100 mg Isoxuprine hydrochloride in 500 ml 5% glucose The infusion rate was 0 4 mg/min for one hour whereby the patients received 25 mg Isoxuprine The infusion was discontinued after one hour and treatment was continued using 10 mg i m every 3 hours for four doses and thereafter 10 mg i m every 4 hours for 48 hours The patients were then given 10 mg every 4 hours this medication was continued at home

Treatment with *ethanol* was started with an infusion solution containing 5% glucose in 1000 ml to which 96 ml 99% ethanol was added The infusion rate was 0 8 ml/min during the first 2 hours and then 0 13 ml/min over 4 hours following which oral treatment was given using 40 ml of cognac every 6 hours This dosage was prescribed also for treatment at home During the period of infusion the patients received 128 ml 99% ethyl alcohol

The parameters recorded during infusion included pulse rate blood pressure and fetal heart rate

Forty three patients were treated with Nylidrin 60 received Isoxuprine 50 received ethanol and 41 placebo The entire series comprised 194 cases treated for imminent premature labour Of these 101 (52%) were primiparae and 93 (48%) were multiparae Eighteen patients were under 20 144 were aged 21 to 30 years and 32 aged 31 to 40 years

Statistical analysis was carried out using the two-dimensional crossbreak measurement Significance was calculated by means of the $\chi^2$ test

## RESULTS

Treatment was considered to have been successful when the premature contractions were arrested and the pregnancy prolonged such that the weight of the newborn was ≥2 500 g or when the premature labour was postponed for at least 7 days and the pregnancy continued until 37th week or longer (Table I) The study revealed that when the weight of the newborn was employed as the criterion a successful result was achieved in 71% of the cases with the placebo product A significantly better result than this was attained with Nylidrin ($p<0.05$) In those cases in which postponement of

Table I *Placebo Nylidrin Isoxuprine and ethanol in treatment of imminent premature labour*

| Drug | Number of patients treated | Successful treatment child ≥2 500 g | | Those with postponement over 7 days who attained the 37th week of gestation or more | |
|---|---|---|---|---|---|
| | | No | % | No | % |
| Placebo | 41 | 29 | 71 | 30 | 73 |
| Nylidrin | 43 | 37 | 86 | 33 | 77 |
| Isoxuprine | 60 | 45 | 75 | 37 | 62 |
| Ethanol | 50 | 35 | 70 | 28 | 56 |

Table II *Successful treatment (newborn ≥2 500 g) in various stages of gestation with placebo Nylidrin Isoxuprine and ethanol*

| Week of gestation at onset of treatment | Placebo | | Nylidrin | | Isoxuprine | | Ethanol | |
|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % |
| 24 to 28 | 6/8 | 75 | 10/12 | 83 | 7/13 | 54 | 9/13 | 69 |
| 29 to 32 | 9/16 | 56 | 10/12 | 83 | 16/21 | 76 | 8/14 | 57 |
| 33 to 36 | 14/17 | 8[illegible] | 17/19 | 89 | [illegible]2/[illegible]6 | 85 | 18/23 | 78 |

delivery for more than 7 days had been successfully achieved the 37th week of gestation was attained in patients receiving placebo in 73% of the group The corresponding result with Nylidrin was 77% whereas the result with the other agents was poorer than with placebo

When the results of treatment are considered in terms of the gestational age at which the imminent premature labour had occurred (Table II) it was observed that the results were the better the further the gestation had progressed at that time

When *the treatment with placebo* failed 3 babies were lost (Table III) When it was successful no babies were lost In addition to uterine contractions there was bleeding in 8 patients in this group in two of whom the treatment failed Five patients (12%) had urinary tract infection during pregnancy

When *the treatment with Nylidrin* failed 4 babies were lost one of them a twin sibling (Table IV) One infant was lost in association with a successful treatment In this case the fetal membranes had ruptured prematurely after 2 weeks of hospital treatment in the 35th week of pregnancy at home Fifteen hours after delivery the baby who weighed 3 400 g succumbed to pneumonia In this group 11 patients were bleeding on admission The treatment failed in two of these cases Three patients (6 7%) had urinary tract infection during pregnancy Appendectomy was performed for one patient during pregnancy

In the cases *treated with Isoxuprine* five babies were lost in connection with therapeutic failures 2 of them were deformed (Table V) No babies were lost when the treatment was successful Haemorrhage occurred in 6 cases in 4 of which the treatment failed Urinary tract infection during pregnancy occurred in 8 patients (12%)

Table III *Therapeutic failures in connection with placebo treatment*

The criterion of successful treatment was the weight of the newborn ≥2 500 g

| Parity | At onset of treatment | | At delivery | | | |
|---|---|---|---|---|---|---|
| | Week of pregnancy | Bleeding | Week of pregnancy | Weight of infant (g) | Length of infant (cm) | Apgar score |
| VI (XI) | 33 | + | 33 | 1 950 | 42 | 9 |
| II | 3[illegible] | – | 33 | 2 280 | 45 | 9 |
| II | 33 | – | 34 | 2 [illegible]00 | 44 | 8 |
| IV | 27 | – | [illegible]7 | 1 150 | 37 | 5/4/1* |
| I (II) | 30 | + | 33 | 1 600 | [illegible] | 3/9 |
| I | 31 | – | 31 | 900 | 33 | 1/[illegible]/0* |
| IV (VI) | 35 | – | 35 | 2 350 | 46 | 9 |
| I | 31 | – | 31 | 1 450 | [illegible] | 2/2/5 |
| I | 28 | – | 36 | 2 420 | 46 | 7 |
| II | 33 | – | 37 | 2 280 | 45 | 9 |
| I | 3[illegible] | – | 34 | 2 450 | 46 | 7 |
| II | 34 | – | 34 | 1 [illegible]00 | 38 | 5/8 |

Apgar scores determined 1/[illegible]/5 minutes after the birth
* Infant lost

Table IV *Therapeutic failures in connection with Nylidrin treatment*

The criterion of successful treatment was the weight of the newborn ≥2 500 g

| | At onset of treatment | | At delivery | | | |
|---|---|---|---|---|---|---|
| Parity | Week of pregnancy | Bleeding | Week of pregnancy | Weight of infant (g) | Length of infant (cm) | Apgar scores |
| I | 28 | + | 28 | 1 000 | ? | 1 |
| I | 32 | – | 32 | 1 760 | 42 | 9/7/8 |
| I | 27 | – | 27 | 1 150 | 38 | 3/5/8 |
| I (II) | 26 | – | 27 | 720 | 29 | 3/2/1 |
| I | 29 | – | 29 | A 1 320 | ? | 5 |
| | | | | B 1 170 | ? | 6[a] |
| II | 33 | + | 33 | 1 800 | ? | 3/6/9 |

Apgar scores determined 1/2/5 minutes after the birth
[a] Infant lost

When *the treatment with ethanol* failed 5 babies were lost (Table VI) When it was successful no babies were lost Bleeding occurred in 6 patients in 4 of whom the treatment was unsuccessful Four patients (8%) had urinary tract infection during pregnancy

*Side effects*

*Among the patients who had received the placebo* 1 patient suffered from nausea (2 4%) The β-sympathomimetics caused hypotension tachycardia dizziness and nausea Mild side effects occurred in 21% of the patients treated with Nylidrin and in 33% of those treated with Isoxuprine When alcohol was used headache and nausea occurred in 10% of the cases

## DISCUSSION

The study revealed that in a double blind trial satisfactory results in prevention of premature labour will be obtained with a placebo The beneficial result is probably caused by bed rest and by

Table V *Therapeutic failures in connection with Isoxuprine treatment*

The criterion of successful treatment was the weight of the newborn ≥2 500 g

| | At onset of treatment | | At time of delivery | | | |
|---|---|---|---|---|---|---|
| Parity | Week of pregnancy | Bleeding | Week of pregnancy | Weight of infant (g) | Length of infant (cm) | Apgar scores |
| I | 35 | + | 35 | 2 180 | 45 | 9 |
| II | 36 | – | 36 | 2 400 | 46 | 9 |
| I | 32 | – | 32 | 2 050 | 45 | 8 |
| II (III) | 29 | – | 29 | 1 700 | 35 | 1/2/2[a] |
| I | 32 | – | 32 | 1 900 | 44 | 7[a] |
| II | 29 | – | 29 | 1 470 | ? | 7/4/6 |
| I | 29 | – | 29 | 1 530 | 41 | 7 |
| I | 26 | + | 26 | 900 | 34 | 7 |
| I (II) | 35 | + | 35 | 2 450 | 47 | 8/10 |
| I | 33 | – | 33 | 2 750 | 45 | 8 |
| II | 26 | – | 26 | 1 040 | 37 | 6/4/6[a] |
| I (IV) | 32 | + | 32 | 1 480 | 37 | 8 |
| II | 25 | – | 26 | 1 185 | ? | 3/5/6 |
| II | 32 | – | 32 | 1 150 | ? | 1/0[a] |
| II (IV) | 30 | – | 30 | 1 500 | ? | 7/3/5 |

Apgar scores determined 1/2/5 minutes after the birth
[a] *Infant lost*

Table VI *Therapeutic failures in connection with ethanol treatment*
The criterion of successful treatment was the weight of the newborn ≥2 500 g

| | At onset of treatment | | At time of delivery | | | |
|---|---|---|---|---|---|---|
| Parity | Week of pregnancy | Bleeding | Week of pregnancy | Weight of infant (g) | Length of infant (cm) | Apgar scores |
| I | 33 | + | 33 | 2 150 | ? | 6/7 |
| I | 31 | − | 32 | A 2 000 | ? | 7 |
| | | | | B 1 300 | ? | 2/2/5 |
| II | 33 | + | 33 | 1 650 | 41 | 9 |
| II | 31 | − | 34 | 1 500 | ? | 7 |
| II | ?4 | − | 25 | 500 | ? | 0* |
| II | 27 | − | ?7 | 760 | ? | 5 |
| III | 34 | − | 35 | 2 ?00 | 48 | 0* |
| I | 31 | − | 37 | ? 450 | 47 | 7/9 |
| I | 30 | + | 30 | 1 500 | ? | 5/7/8* |
| I | 9 | + | 29 | 1 140 | ? | 1/6 |
| I | 3? | − | 34 | ? 330 | 45 | 9 |
| II | ?5 | − | 25 | 840 | 34 | 2* |
| I | 33 | − | 33 | 1 980 | 45 | 1/7 |
| II | 28 | − | ?8 | 1 070 | ? | 7 |
| II | 31 | − | 31 | 1 900 | ? | 9 |

Apgar scores determined 1/?/5 minutes after the birth
Infant lost

the reassuring feeling experienced by the patient as a consequence of seemingly efficient attempts to treat her It has also been observed that a 5% glucose infusion alone has a slight but prolonged *inhibiting effect on uterine activity (1) In our* study only Nylidrin proved statistically more efficient than the placebo Isoxuprine has a somewhat similar but weaker effect In this study alcohol did not prevent premature labour

Isoxuprine treatment has been successful in 42% when the criterion employed was a birth weight of at least 2500 g (2) and imminent premature labour in 25 patients could thus be successfully postponed for 1 to 12 weeks in 7?% (5) In a control group of the same size treated with bed rest sedative and antispasmodic drugs 19 patients gave birth to a child within 24 hours and in no case was it possible to postpone delivery for over one week The sympathomimetic agent the test product TV 399 when used for treatment of premature labour resulted in the delivery of a viable child in approximately 95% (10)

When using alcohol delivery was postponed at least until the 37th week of gestation in 52% (6 11) or in 60% (15) All of the above trials were performed without an adequate control material Twenty patients in 15 of whom the membranes were intact were treated with alcohol infusions (7) With the exception of one patient premature delivery occurred within 2 to 3 days In the study performed by Zlatnik & Fuchs (17) 21 imminent *premature labours* were treated with alcohol The delivery was postponed for at least 3 days in 17 cases (81%) A control group of the same size was treated with a glucose drip with successful results in 8 cases (38%)

Since it is only possible to prevent a proportion premature deliveries it is important to know what effect the agents used in treatment will eventually have on the newborn infant As a rule the $\beta$-sympathomimetic drugs have been found to be safe for the fetus (10) and sometimes beneficial in cases of asphyxia (3) When alcohol was administered as an intravenous drip to women in labour 90 minutes to 3½ hours prior to delivery the condition of the newborn was generally good with the exception of a few cases of transient muscular hypotension A depressant action upon respiration was not observed The threat of alcohol induced central nervous system depression with subsequent respiratory and circulatory alterations is however probably greater in cases of the premature neonate (13) Horiguchi et al (8) observed that alcohol did not suppress uterine contractions

On the basis of the present study it is clear that alcohol even when administered intravenously in fairly large doses did not result in better therapeutic results than the placebo Since in addition it may be dangerous to the newborn especially in cases of prematurity its use for prevention of premature labour is not recommended On the other hand it seems possible that the β-sympathomimetic drugs especially after further development of products with fewer side effects upon the mother may well offer a more efficient means of preventing premature delivery The efficacy of these drugs is quite understandable because there are indications suggesting the prevalence of β sympathotonus during normal pregnancy This has been demonstrated by the changes occurring in the regulation of blood pressure (4) as well as by the fact the excretion of cyclic AMP into the urine is considerably increased during gestation (14) In those cases where uterine contractions are generally increased as in toxaemia of pregnancy the excretion of cyclic AMP is significantly decreased (12) Thus it is possible that the beneficial effect of β sympathomimetic drugs is not only due to suppression of the uterine muscular activity but also to the restoration and maintenance of the changes in haemodynamics which occur during pregnancy and can be regarded as beneficial to it

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*Submitted for publication July 6 1973*

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Acta Obstet Gynec Scand 54 101–103 1975

# EFFECT OF CENTRIFUGATION ON AMNIOTIC FLUID PHOSPHOLIPID RECOVERY

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*Abstract* Amniotic fluid lecithin and sphingomyelin were determined quantitatively in 33 samples obtained through out the last trimester Each sample was divided into three parts and each part was centrifuged at different relative forces prior to extraction It is shown that centrifugation always removes considerable amounts of both lecithin and sphingomyelin from the supernatant towards the end of pregnancy but very little at the beginning of the last trimester The fatty acid composition of amniotic fluid lecithin studied by gas liquid chromatography indicate that surface active lecithin is lost by centrifugation of the fluid prior to extraction

Several reports have appeared recently on the subject of amniotic fluid lecithin and sphingomyelin determination as an index of fetal pulmonary maturity The variation in the centrifugal forces employed by the authors have tended to make interpretation and comparison of the lecithin and sphingomyelin concentrations difficult Centrifugation forces have ranged from uncentrifuged (1 2) to 5400 r p m for 5–10 minutes (4 5 6 7) Those workers who are using Gluck s procedure (4 5) may experience difficulty since the *g* minute force will vary at the same centrifugation speed depending on the diameter of the centrifuge and also because different centrifugation speeds have been reported

The reason for centrifugation of amniotic fluid prior to lipid extraction has been removal of cells and debris The present investigation was carried out in order to study the effect of centrifugation on the lecithin and sphingomyelin content in the supernatant as compared to uncentrifuged amniotic fluid

## MATERIALS AND METHODS

Amniotic fluid was obtained in all cases by trans abdominal paracentesis Amniotic fluid contaminated with blood or meconium was discarded Determinations were carried out as previously outlined (5) after dividing each sample into three parts These were tested un centrifuged centrifuged at 900 $g \times 10$ minutes and at 12800 $g \times 10$ minutes Thirty three samples of amniotic fluid were studied and the available amount allowed a total of 76 measurements of lecithin sphingomyelin and *L/S ratio respectively In each instance the amniotic* fluid was carefully mixed before the sample was pipetted off

The fatty acid composition of amniotic fluid was studied by gas liquid chromatography in 10 samples at 34–40 weeks gestation Each sample was divided into two parts Determinations were carried out on the un centrifuged part the supernatant after centrifugation at 1980 $g \times 5$ minutes (4000 r p m for 5 minutes) as well as on the pellet This centrifugation speed has been advocated by Gluck (4) and was chosen for that reason The amniotic fluid was extracted and developed by thin layer chromatography as stated above After identification the lecithin spot was eluted and fatty acid methyl esters were prepared by the method described by Glass (3) The quantity of each fatty acid is calculated in percent of all six fatty acids Lecithin may have any combination of two of these

## RESULTS

The amniotic fluid lecithin and sphingomyelin concentration as well as the L/S ratios were grouped within the periods of gestational age in which they were obtained Averages were taken for each *g* minute force employed and these were compared for each gestational group The results are illustrated in the figures for uncentrifuged amniotic fluid (I) after centrifugation at 900 $g \times 10$ minutes (II) and at 12800 $g \times 10$ minutes (III)

Fig 1 illustrates the lecithin concentrations recorded Towards the end of pregnancy moderate centrifugation (900 $g \times 10$ minutes) removes approximately 50% of the lecithin Moderate centri

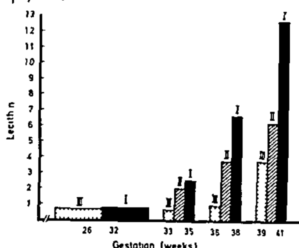



*Fig 1* Average concentrations of lecithin in uncentrifuged amniotic fluid (I) after centrifugation at 900 $g \times 10$ minutes (II) and 12800 $g \times 10$ minutes (III) for each gestational period

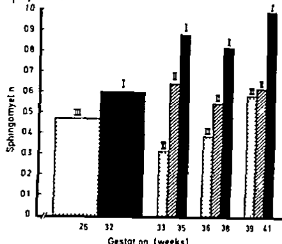



*Fig 3* Average concentrations of sphingomyelin in uncentrifuged amniotic fluid (I) after centrifugation at *900 $g \times 10$ minutes (II) and 12800 $g \times 10$ minutes (III)* for each gestational period

fugation removes less at *earlier gestations and at the* beginning of the last trimester relatively forceful centrifugation (12800 $g \times 10$ minutes) removes *negligible amounts only*

Fig 2 shows that moderate centrifugation reduces the L/S ratio less than the lecithin concentration towards the end of pregnancy From 26–35 weeks of gestation moderate centrifugation does not alter the L/S ratio appreciably Centrifugation at 12800 $g \times 10$ minutes however reduces the L/S ratio 40–60% except at the earliest part of the last where there is no change

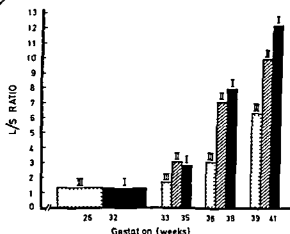



*Fig 2* Averages of calculated L/S ratios in uncentrifuged amniotic fluid (I) after centrifugation at *900 $g \times$* 10 minutes (II) and 12800 $g \times 10$ minutes (III) for each gestational period

*The alterations in the L/S ratio produced by* centrifugation of the amniotic fluid with different relative forces can be explained by examining the effect that centrifugation has on the sphingomyelin concentration illustrated in Fig 3 Towards the end of pregnancy moderate centrifugation reduces the sphingomyelin concentrations somewhat less than the lecithin concentrations thereby producing a small but definite fall in the L/S ratio From 26–35 weeks gestation moderate centrifugation reduces sphingomyelin and lecithin nearly in equal proportions and therefore does not alter the L/S ratio appreciably The effect of centrifugation at 12800 $g \times 10$ minutes on the sphingomyelin concen*tration is relatively less pronounced than for* lecithin which in turn brings the L/S ratio down considerably

*Table I illustrates the fatty acid pattern in one of* the ten samples determined There is no appreciable difference in the fatty acid composition of un centrifuged amniotic *fluid supernatant after centri*fugation or the pellet

There is general agreement that lecithin with the highest surface activity contains two saturated fatty acids particularly palmitic It may be seen that approximately 75% of the fatty acids (myristic palmitic and stearic) are saturated in all three parts of the amniotic fluid studied The same trend appeared in 10 consecutive samples More specifically the proportion of palmitic acid found in the supernatant after centrifugation was not increased as

Table 1 *Fatty acid composition expressed in percent for each fatty acid in uncentrifuged amniotic fluid after centrifugation at 1980 g×5 minutes and in the pellet*

| Fatty acid composition in % | Myristic | Palmitic | Palmit oleic | Stearic | Oleic | Linoleic |
|---|---|---|---|---|---|---|
| Uncentrifuged | 2 8 | 69 7 | 4 6 | 4 2 | 14 9 | 3 9 |
| 1980 g×5 min | 2 1 | 70 9 | 3 9 | 5 1 | 1? 7 | 5 4 |
| Pellet | 3 2 | 67 0 | 4 9 | 6 7 | 15 2 | 3 1 |

compared to the pellet or in uncentrifuged amniotic fluid itself Centrifugation therefore removes surface active lecithin

## DISCUSSION

The rationale behind centrifugation of amniotic fluid used for surfactant determination has been *removal of cells and debris This study shows that* centrifugation has a marked effect upon lecithin recovery and less upon the recovery of sphingomyelin This removal of phospholipid is insignificant at the beginning of the last trimester but increases with increasing gestational age and is at its maximum towards the end of pregnancy It is a so dependent upon the relative force of centrifugation used The rate of removal of phospholipid from the supernatant increases with the relative force used but it is not uniform nor is it the same for lecithin and for sphingomyelin Removal of lecithin and *sphingomyelin in different proportions will there*fore change the L/S ratio Fatty acid studies show that there can be very little difference in the type of lecitin found in the supernatant or in the pellet after centrifugation showing that this procedure removes surface active lecithin and is of little use in selectively removing phospholipid from other sources Since alterations in the centrifugal forces will change both lecithin concentration and the L/S ratio we have stopped using this procedure prior to amniotic fluid extraction

Considering the centrifugation forces used in this series of experiments and the different ability to remove phospholipids from amniotic fluid at early and late gestations even 12800 $g \times 10$ minutes is relatively modest in terms of removing very small particle matter This suggests that the amniotic fluid lecithin must be part of or bound to relatively large particles in the amniotic fluid

## CONCLUSIONS

The present data show that centrifugation of amniotic fluid removes surface active lecithin This effect increases with increasing gestational age and the g force employed Removal of lecithin and sphingomyelin in different proportions alters the *L/S* ratio Surface active lecithin as demonstrated by fatty acid determinations adheres to or consists of large particles which precipitate on centrifugation

## ACKNOWLEDGEMENTS

We are indebted to Prof M Seip Department of Pediatrics Prof K Bjøro Department of Obstetrics and *Gynecology Rikshospitalet the Hartford Foundation* Wisconsin USA and Norske Kvinners Sanitetsforening for making this study possible

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*Submitted for publication Oct 16 1973*

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Acta Obstet Gynec Scand 54 105–111 1975

# STUDIES IN CHOLESTASIS OF PREGNANCY

## II *Serum Lipids and Lipoproteins*

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*Abstract* Forty-one pregnant women with pruritus in whom cholestasis was verified by the presence in their serum of an abnormal lipoprotein lipoprotein X (LP X) were divided into two clinical groups pruritus gravidarum (PG) ($n=20$) and hepatosis of pregnancy (HP) ($n=21$) in relation to serum bilirubin (below and above 1 2 mg/100 ml respectively) and/or SGOT SGPT (below and above 50 units/l respectively) In HP but not in PG serum lipids i e cholesterol phospholipids triglycerides pre $\beta$-lipoproteins (very low-density lipoproteins) and low-density lipoproteins were increased and high-density lipoproteins decreased when compared with suitable controls Serum lipids were elevated in proportion to the derangement in the liver function tests alkaline phosphatase SGOT and SGPT The occurrence of LP X was inversely related to HDL cholesterol suggesting a causal relationship between HDL lipid metabolism and the presence of LP X Serum TIBC Simplastin A and serum iron were elevated in HP in relation to the degree of deterioration of liver function tests Some of these changes in serum in cholestatic pregnancy may partially (serum triglycerides and pre $\beta$-lipoproteins) or completely (TIBC and Simplastin A) be explained by an enhanced estrogen influence in promoting increased liver lipid/protein metabolism

Normal pregnancy is accompanied by elevated serum lipid levels A progressive increase in serum triglycerides throughout pregnancy is particularly marked Serum cholesterol reaches its maximum level about 2 months before term (10 25 38)

Lipids in serum are transported as lipoproteins Four classes of serum lipoproteins are present in human serum Each of these has a characteristic size density and electrophoretic mobility The triglyceride rich *chylomicrons* transport exogenous fat from the intestine The triglyceride rich and cholesterol-containing *very low-density lipoproteins* (VLDL) also knows as the pre $\beta$ lipoproteins because of their electrophoretic mobility carry endogenous triglycerides The cholesterol rich *low density lipoproteins* (LDL) which have the electrophoretic pattern of $\beta$ globulins ($\beta$-lipoproteins) transport the major portion of serum cholesterol Finally there are the *high-density lipoproteins* (HDL) with a high control content of cholesterol and phospholipids which migrate as $\alpha$-globulins ($\alpha$-lipoproteins) on electrophoresis

The change in serum lipids and lipoproteins during pregnancy are considered to be hormonally induced (9 12 21 22) During normal pregnancy the levels of VLDL LDL and HDL are increased (10 12 41) The administration of ethinylestradiol to oophorectomized women causes an increase in VLDL and HDL with a concomitant decrease in LDL (15)

It has been stated that an increase in serum triglycerides is a characteristic and sensitive indicator of estrogen administration (12) However Lebech & Borggaard (23) were unable to induce increased serum triglycerides in fertile women giving small doses of 17 $\beta$-estradiol and estriol combined with nor-ethisterone Pruritus gravidarum (PG) and hepatosis of pregnancy (HP) are considered to be different stages of the same cholestatic syndrome In this syndrome itching is often the first and foremost symptom (19) The cause of this syndrome is unknown but the available evidence suggests that an abnormal influence of estrogen on liver cell metabolism is present (1)

Cholestatic conditions generally cause characteristic changes in serum lipids with increases in free cholesterol and phospholipids which mainly are due to the simultaneous appearance of an abnor

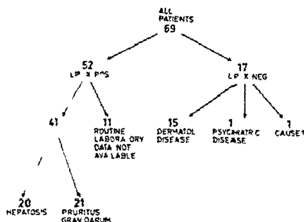



*Fig 1* Patient series

mal lipoprotein lipoprotein X (LP X) within LDL density class (31 34) In a recent report (19) evidence has been presented to show that the occurrence in serum of LP X can be used as a diagnostic criterion in cholestasis of pregnancy

Here pregnant women with cholestasis verified by the presence of LP X in serum and designated as PG or HP have been studied in relation to liver function tests serum lipids lipoproteins and hematological variables

## MATERIALS AND METHODS

Consecutive patients with pruritus during pregnancy ferred to a pruritus out patient clinic at the Mater l Welfare Unit have been studied The patients were ferred from a community corresponding to a population of approximately 200 000 Sixty nine patients were admitted during a period of 15 months In 52 cases the presence in serum of LP X was verified by a modified immuno diffusion technique (19) Of these 11 were lacking in clinical information or laboratory data leaving 41 LP X positive cases (21 with PG and 20 with HP) for study (Fig 1) Among the LP X negative 15 women suffered from dermatological conditions likely to cause pruritus one had psychiatric symptoms which could explain the itching and in one case no cause was found

Twenty healthy pregnant women randomly selected from the same Maternal Welfare Unit served as controls

Fasting venous blood samples were taken in the morning at the time of the clinical examination These were analysed for liver function tests serum total bilirubin (normal <1 2 mg/100 ml) alkaline phosphatase (normal <8 Buch units) SGOT (normal <17 Units/l) and SGPT (normal <17 Units/l) Serum triglycerides were determined according to Carlson (7) total cholesterol by the method of Cramér & Isacsson (8) and phospholipids as described by Svanborg & Svennerholm (37) The cholesterol content (24) of high density lipoproteins (HDL) was measured after precipitation of the very low density lipoproteins (VLDL) and low density lipoproteins (LDL) with manganese chloride and heparin (5) The cholesterol content of LDL was estimated from the following equation *LDL cholesterol=serum cholesterol−HDL cholesterol*−serum triglycerides×*F* For *F* the value 0 20 was used for serum triglycerides<180 mg/100 ml and the value 0 25 for serum triglycerides>180 mg/100 ml (13) The semi-quantification of LP X was performed as described earlier (19) by a modified immuno diffusion technique Lipoprotein electrophoresis was performed on 1% agarose gel in barbital buffer (pH 8 6) ionic strength 0 05 Lipoprotein bands were stained with a mixture of Oil Red O and Fett Rot B (Ciba) and were visually evaluated (14)

Serum iron serum total iron binding capacity (TIBC) and Simplastin A were determined according to standard methods and folic acid in whole blood and serum according to Hansen (16)

### *Statistical methods*

Conventional methods were used for the calculation of means standard error of means and correlation coefficients Some of the variables showed skewed distribution and hence non parametric methods were used in the significance tests Differences between groups were analysed by the median test and the association between two variables by the chi square test

The relationship between serum triglycerides and SGOT was found to be non linear and therefore a second-degree function was fitted to the observations by the method of least squares and the degree of dependence was measured by the coefficient of determination (*D*) (3)

Table I *Liver function tests in pruritus gravidarum (PG) (n=20) and hepatosis of pregnancy (HP) (n=21)* Mean ±S E M

| | *n* | Bilirubin (mg/100 ml) | Alkaline phosphatase Buch units | SGOT (U/l) | SGPT (U/l) |
|---|---|---|---|---|---|
| PG | 0 | 0 5±0 1 | 9 0±1 0 | 15 8±2 1 | 12 9±2 5 |
| HP | 21 | 1 2±0 2 | 17 2±0 8 | 64 8±6 4 | 86 9±10 5 |

Table II *Serum lipids and lipoproteins in pruritus gravidarum (PG n=20) hepatosis of pregnancy (HP n=?1) and control series (n=20)*

Mean ±S E M N S =not significant

| | $n$ | Cholesterol (mg/100 ml) | Phospholipids (mg/100 ml) | Triglycerides (mg/100 ml) | HDL cholesterol (mg/100 ml) | LDL cholesterol (mg/100 ml) | LP X (arb units) |
|---|---|---|---|---|---|---|---|
| I PG | 0 | 62±9 | 30?±10 | 198±18 | 74±5 | 143±9 | 2 1±0 1 |
| II HP | ?1 | 3?6±16 | 356±18 | 266±1? | 60±3 | 199±13 | 3 3±0 2 |
| III Control series | 20 | ?65±8 | 283±7 | 180±13 | 77±3 | 147±8 | – |
| I vs III | $p<$ | N S | N S | N S | N S | N S | – |
| II vs III | $p<$ | 0 01 | 0 01 | 0 001 | 0 001 | 0 01 | – |

Measured value Estimated value

## RESULTS

*Liver function tests* (Table I)

In PG only the mean value of alkaline phosphatase was above the normal value In HP the mean values for all variables studied were above the normal limits By definition serum bilirubin SGOT and SGPT were higher in HP than in PG

*Serum lipids and lipoproteins* (Table II)

Serum lipids were not elevated in PG In HP all lipid variables were elevated on comparison with the control series

In spite of the elevation of serum lipid levels in HP HDL cholesterol was lower than in the control series ($p<0\ 001$) Estimated LDL cholesterol was elevated in HP ($p<0\ 01$) concomitant with the occurrence of L P X in serum LP X was higher in HP than in PG

Lipoprotein electrophoresis revealed in PG an increase in pre $\beta$ lipoproteins and $\alpha$ lipoproteins while in HP pre $\beta$ lipoproteins and $\beta$ lipoproteins (due to the occurrence of LP X) were more pronounced

*Hematological variables* (Table III)

TIBC was elevated ($p<0\ 05$) in PG as compared with levels in normal pregnant women In HP serum iron ($p<0\ 01$) and TIBC ($p<0\ 001$) were higher than in normal pregnancy The level of folic acid was similar in PG and HP Simplastin A was higher ($p<0\ 05$) in HP

*Correlations between serum lipids and liver function tests* (Table IV)

In the combined group of PG and HP (41) the values of alkaline phosphatase correlated with all serum lipid variables cholesterol ($r=0\ 44$ $p<0\ 05$) phospholipids ($r=0\ 35$ $p<0\ 05$) and triglycerides ($r=0\ 45$ $p<0\ 01$) SGOT and SGPT correlated with serum cholesterol ($r=0\ 41$ $p<0\ 05$ and $r=0\ 37$ $p<$

Table III *Hematological data in PG (n=18) HP (n=19) and in normal pregnancy (n=15)*

Mean ±S E M N S =not significant

| | | Serum iron (g/100 ml) | TIBC (g/100 ml) | Folic acid whole blood (ng/ml) | Folic acid serum (ng/ml) | Simplastin A (%) |
|---|---|---|---|---|---|---|
| I PG | | 106±12 | 534±_1 | 1 2±25 | 6 7±1 3 | 125±9 |
| II HP | | 147±14 | 59_±30 | 1_4±11 | 6 9±0 7 | 157±8 |
| III Normal pregnancy | | 100±8 | 464±18 | – | – | – |
| I vs II | $p<$ | – | – | N S | N S | 0 05 |
| I vs III | $p<$ | N S | 0 05 | – | – | – |
| II vs III | $p<$ | 0 01 | 0 001 | – | – | – |

Data from B Svanberg Personal communication

Table IV *Correlations between liver function tests and hematological data serum lipids and lipoproteins in the combined groups of PG and HP (n=41)*

* $p<0.05$ ** $p<0.01$ ** $p<0.001$ N S =not significant

| | Bilirubin | Alkaline phosphatase | SGOT | SGPT |
|---|---|---|---|---|
| Triglycerides | N S | $r=0.45$** | $D=0.24$ | $r=0.33$* |
| Cholesterol | N S | $r=0.44$* | $r=0.41$ | $r=0.37$* |
| Phospholipids | N S | $r=0.35$* | N S | N S |
| LP X | $r=0.52$** | $r=0.40$* | $r=0.72$ * | $r=0.71$ |
| Serum iron | N S | $r=0.50$** | $r=0.48$ * | $r=0.44$ * |
| TIBC | N S | $r=0.42$ | N S | N S |
| Simplastin A | N S | $r=0.40$ | N S | N S |

0 05 respectively) but not with phospholipids SGPT was also correlated to triglycerides ($r=0.33$ $p<0.05$)

The relation between serum triglycerides and SGOT in the combined groups of PG and HP was found to be non linear ($D=0.24$ $p<0.05$) and the function $y=149.9042+3.5051x-0.0238x^2$ fitted the observations Maximum serum triglycerides value in this non linear function was at SGOT=73 6± 22 8 (standard error of the estimation) (Fig 2)

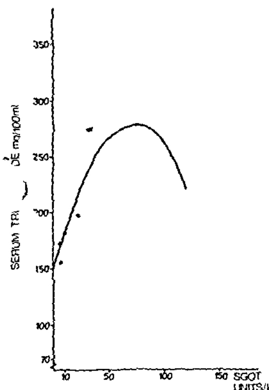



*Fig 2* Relationship between serum triglycerides and SGOT in the combined group of pruritus gravidarum (PG) and hepatosis of pregnancy (HP) The non linear (parabolic) function $y=149.9042+3.5051-0.0238x^2$ fitted the observations *Maximum y value (serum triglycerides)* was at *x* value (SGOT) 73 6±22 8 (±standard error *of the estimation)*

LP X was a good index of liver dysfunction as the amounts of LP X in serum correlated with the concomitant values of all liver function tests serum bilirubin ($r=0.52$ $p<0.001$) alkaline phosphatase ($r=0.40$ $p<0.01$) SGOT ($r=0.72$ $p<0.001$) SGPT ($r=0.71$ $p<0.001$)

Serum iron correlated with alkaline phosphatase ($r=0.50$ $p<0.01$) SGOT ($r=0.48$ $p<0.01$) and with SGPT ($r=0.44$ $p<0.01$) TIBC and Simplastin A correlated with alkaline phosphatase ($r=0.42$ $p<0.05$ and $r=0.40$ $p<0.01$ respectively)

*Correlations between serum lipids and lipoproteins* (Table V)

The values of serum lipids were highly interrelated

LP X varied positively with serum triglycerides ($r=0.38$ $p<0.05$) and in opposite direction with HDL cholesterol ($r=0.32$ $p<0.05$)

Table V *Correlations between serum lipids and lipoproteins in the combined groups of PG and HP (41)*

* $p<0.05$ ** $p<0.01$ * $p<0.001$ N S =not significant

| | Triglycerides | Phospholipids | HDL cholesterol |
|---|---|---|---|
| LP X | $r=0.38$ | N S | $r=-0.3?$ |
| Cholesterol | $r=0.59$ * | $r=0.88$ | N S |
| Phospholipids | $r=0.53$ * | – | N S |

## DISCUSSION

In HP (but not in PG) serum cholesterol phospholipids and triglycerides were higher than in normal pregnancy The levels of serum cholesterol (2 26 29) and phospholipids (2 29) were in agreement with earlier findings in cholestasis of pregnancy The increase in serum cholesterol and phospholipids in HP was accompanied by an increase in LDL and $\beta$ lipoproteins and the presence of an abnormal serum lipoprotein LP X This is what might be expected since LP X the characteristic findings in cholestasis has the density of LDL and the electrophoretic mobility of $\beta$ lipoproteins (31)

Although all serum lipid variables were increased HDL cholesterol was lower in HP (but not in PG) than in normal pregnancy The reduction in HDL lipids was also apparent from the decrease in $\alpha$-lipoproteins on lipoprotein electrophoresis These findings in cholestasis of pregnancy are in agreement with earlier studies utilizing quantitative lipoprotein electrophoresis (2) and immunological methods (11) It has been suggested that the reduction in HDL lipids in cholestasis is an expression of impaired lipid binding capacity of the major protein moiety of HDL the Apo-lipoprotein A (33) In the present study the reduction in HDL cholesterol was related to the occurrence of LP X A similar negative relationship between the changes in HDL lipids and LP X has earlier been suggested in cholestasis (27) These findings might further suggest a relationship between HDL metabolism and the occurrence of LP X

However the observed increase in total serum cholesterol and phospholipids cannot be attributed to a redistribution of cholesterol and phospholipids among lipoprotein fractions In cholestasis elevated serum cholesterol and phospholipids are a regular finding The results of animal experiments involving the ligation of the biliary duct in rats have suggested that the cholesterol increase is due to increased cholesterol synthesis in the liver (40) The elevated serum phospholipids might also be due to increased hepatic synthesis as the addition of cholic acid to liver slices in vitro enhances the incorporation of precursors into lecithin (6)

Serum triglycerides were high in HP (but not in PG) There has been no previous convincing demonstration of this in cholestasis of pregnancy In the series of Svanborg et al (39) no conclusive data on serum triglycerides were given In the fasting state an increased level of serum triglycerides usually indicates an increase in VLDL In the present study in both HP and PG pre $\beta$-lipoproteins (VLDL) were increased Increased concentrations of pre $\beta$-lipoproteins and serum triglycerides do not appear to be a common finding in other cholestatic conditions (30) In the present study serum triglycerides showed a successive increase in relation to moderately elevated (<50 Units/l) values of SGOT (Fig 2) while with a further increase in SGOT serum triglycerides levelled off and with even higher SGOT values serum triglycerides levels fell The initial moderate increase in SGOT together with increasing triglyceride levels is similar to the effects of estrogens on SGOT (28) and serum triglycerides (15) The maximum serum triglyceride value in the non linear function was at SGOT value 73 6 units/l The standard error of this estimation was 22 8 suggesting that the apex of the serum triglyceride curve would correspond to a minimum SGOT value of 49 7 units/l This calculation suggests that the SGOT value of 50 units/l can discriminate between PG and HP

The elevated TIBC and Simplastin A appear to be new findings in cholestasis of pregnancy Elevated serum iron has been found before (18 43) The liberation from necrotic iron rich liver cells (4 13) would explain the increase in serum iron (cf the correlations between serum iron and SGOT and SGPT) but not the increase in TIBC and Simplastin A The elevation in TIBC and Simplastin A may have a common cause as these variables appeared to be correlated ($r=0\ 35$ $p<0\ 05$) Estrogens enhance protein synthesis in the liver (cf 34) and would be expected to increase serum proteins (9) such as Simplastin A (composed of coagulation factors II VII and X) as well as TIBC (transferrin) (22 42)

The present data appear to give further indirect support to the proposition that an increased sensitivity to estrogen or its metabolites in the liver participates in the development of cholestasis in pregnancy In a forthcoming publication we will present further evidence to this effect (20)

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*Submitted for publication October 17 1973*

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Acta Obstet Gynec Scand 54 113–118 1975

# THE HAZARDS OF VACUUM ASPIRATION IN LATE FIRST TRIMESTER ABORTIONS

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*Abstract* The incidence of somatic complications in connection with legal termination of pregnancy by vacuum aspiration was analysed in 1 123 hospital patients Special attention was paid to complication rates in relation to gestational age It was found that the incidence of major uterine haemorrhage increased with gestational period being unexpectedly high in the 12th week When anaesthesia was supplemented with halothane there was a significantly higher incidence of uterine haemorrhage than when this anaesthetic was avoided The results indicate that strict principles for the operation procedure are mandatory to reduce blood loss and other complications It is suggested that the end of the 12th week should not be considered as a magic time limit for vacuum aspiration but that the policy should aim at early intervention preferably before the end of the 10th week In the event of late first trimester abortions or border line cases it is of advantage to administer prostaglandin extra amniotically for pre-operative dilatation of the cervix

Termination of pregnancy by vacuum aspiration (VA) is considered to be a comparatively simple and safe procedure which in the majority of cases does not require admission to hospital It is evident however that the complication rate increases markedly with increasing gestation period This is particularly true in relation to the incidence of uterine haemorrhage Many authors do not define the method by which the amount of bleeding was estimated and do not relate the incidence of this complication to period of gestation It is also clear that recorded complications in terms of temperature rise and endometritis tend to increase if the patients are supervised in hospital or by follow up examination after the operation

The present study was undertaken to analyse in particular the complication rate in connection with VA related to week of pregnancy Also an attempt was made to evaluate morbidity during the two-month period following operation

## MATERIAL AND METHODS

The case material consists of 1 123 women in the 6th–15th week of gestation as calculated from the first day of the last menstrual period These cases comprise all legal abortions carried out by VA during the period 1967–1971 at the Karolinska Hospital Out of these patients 46 women were primigravidae and 661 multigravidae The distribution of the cases in relation to gestational age and parity is shown in Fig 1 It should be emphasized that more than 50 % of the patients were in the 1 th week of pregnancy or later The operation was performed under general anaesthesia which included i v enibomal sodium (Narkotal Astra Sweden) and inhalation of a mixture of oxygen–nitrous oxide In 55 % of the cases (619 out of 1 123 women) the anaesthesia was also supplemented with halothane Paracervical block (20 ml 0.5 % Citanest without adrenaline (Astra Sweden)) was administered in addition to facilitate dilatation of the cervix An electrical vacuum pump and metal cannulas (8 10 or 12 mm outer diameter) were utilized for the evacuation of the uterine cavity following dilatation of the cervix by Hegar dilators The negative pressure applied usually corresponded to 0 4 kg per cm² After evacuation of the uterine contents the procedure was completed by routine check curettage Methergine (Sandoz) (0 2 mg) was administered i v during the operation The uterine contents and the blood lost during the operation were collected The total amount was measured and recorded in each individual case (without correction for the fetoplacental tissues and the amniotic fluid volume)

The patients were kept in hospital until the following morning or longer if any complications were suspected In the latter cases prolongation of the hospital stay may have served as some guarantee that a post-operative temperature rise or other symptoms did not escape attention The patients were interviewed and examined at a follow up visit within the next two month period If

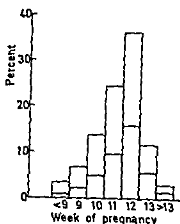



*Fig 1* Distribution of the cases in relation to week of pregnancy The shadowed area indicates the proportion of primigravid women

so required the patients were checked at additional clinic visits or were admitted to the hospital depending upon the nature of the complication

## RESULTS

### *Uterine perforation*

The incidence of uterine perforation in the whole series was 0 45% The occurrence of this complication as related to week of pregnancy is shown in Table I There were 5 incidences of perforation and all occurred in the 11th–12th week The diagnosis was confirmed at laparotomy and hysterectomy had to be performed in one of the patients r out of the 5 patients were primi gravidae

### *Uterine haemorrhage during operation*

To evaluate the incidence of uterine haemorrhage the series was divided into classes depending on the amount of blood loss at operation Since it has been shown previously that halothane anaesthesia is associated with increased uterine haemorrhage (3) the material was also analysed with regard to

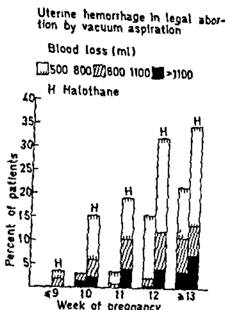



*Fig 2* Incidence of uterine haemorrhage associated with legal abortion by vacuum aspiration related to week of pregnancy Approximately 50% of the patients received general anaesthesia supplemented with halothane inhalation The bars not indicated by *H* represent patients who were not given halothane during the operation

type of anaesthesia Fig 2 shows the percentage distribution of the classes with and without halothane as calculated for each week of pregnancy There is a progressive increment in the incidence of heavy bleeding along with increasing gestational age In the group where halothane had not been utilized there was a significantly lower incidence of uterine haemorrhage than in the halothane group In the 9th week of pregnancy or earlier none of the patients had a blood loss of 500 ml or more and in the 10th and 11th week approximately 3% exceeded the 500 ml limit However there was a dramatic change in the 12th week in that as many as 16 2% of the patients had a bleeding of 500 ml or more and this tendency was even more marked in the 13th week group

The patients who received halothane had a stepwise increment in the incidence of large uterine haemorrhage increasing with each week of pregnancy As much as 15% of the patients in the 10th week and over 30% of the patients in the 12th week had a blood loss exceeding 500 ml

There was no difference in the incidence of bleeding (>500 ml) between primi gravid and multi gravid women

Table I *Incidence of uterine perforation at different gestational weeks*

| | Gestation (weeks) | | | | | |
|---|---|---|---|---|---|---|
| | ≤9 | 10 | 11 | 12 | ≥13 | Total |
| Uterine perf / total no | 0/118 | 0/156 | ?/274 | 3/410 | 0/165 | 5/1123 |
| Per cent | 0 | 0 | 0 73 | 0 73 | 0 | 0 45 |

Table II *Repeat curettage*

| | Gestation (weeks) | | | | |
|---|---|---|---|---|---|
| | ≤9 | 10 | 11 | 12 | ≥13 |
| Repeat currettage/ total no | 3/118 | 1/156 | 4/274 | 5/410 | 3/165 |
| Per cent | 2 5 | 0 6 | 1 5 | 1 2 | 1 8 |

### *Repeat curettage*

The group requiring repeat curettage included patients admitted to the hospital within two months due to late uterine haemorrhage or other symptoms of retained products of conception The over all incidence corresponded to 1 4% Table II shows the percentage distribution of this complication as related to week of pregnancy There was no definite difference in this respect between the gestational weeks

### *Post abortion infection*

Pelvic infection during the post operative stay in the hospital included patients with clinical symptoms and signs of endometritis salpingitis and/or parametritis or a temperature rise to 38°C or more on two consecutive occasions (early post abortion infection) Patients re admitted to the hospital with clinical symptoms of pelvic infection were recorded in a separate group (late post abortion infection) It should be emphasized that the latter group also included women with suspicion of pelvic infection and not only patients with a well defined and definite diagnosis However the group with suspicion of pelvic infection included patients complaining of symptoms severe enough to motivate acute medical attention and admittance to the hospital upon judgement of the gynecologist in charge

Table III *Occurrence of early and late pelvic infection*

| | Gestation (weeks) | | | | |
|---|---|---|---|---|---|
| | ≤9 | 10 | 11 | 12 | ≥13 |
| Early infection/total no | 1/118 | 7/156 | 6/274 | 11/410 | 6/165 |
| Per cent | 0 8 | 4 5 | | 2 7 | 3 6 |
| Late infection/total no | 7/118 | 7/156 | 7/274 | 24/410 | 5/165 |
| Per cent | 5 9 | 4 5 | 2 5 | 5 9 | 3 0 |

Table IV *Complication rate as related to physician experience*

| Physician category (years of training) | No of cases | No of cases with complications | % |
|---|---|---|---|
| (*a*) <1 | 290 | 66 | 22 8 |
| (*b*) 1–3 | 250 | 69 | 27 6 |
| (*c*) ≥4 | 580 | 118 | 20 3 |

On the basis of these definitions the incidence of post abortion pelvic infection was rather high ranging between 0 8–4 5 (early infection) and 2 6–5 9% (late infection) (Table III) The over all figure corresponded to 7 2% There was no clear-cut correlation to gestational age

### *Physician experience*

The complication rate was also analysed with regard to physician experience The staff members who are all employed on a full time basis were classified as follows (*a*) phsyicians with less than 1 year of experience within obstetrics and gynecology (*b*) 1–3 years and (*c*) 4 years or more

If a less experienced physician started the operation but called for assistance by a more experienced colleague to complete the procedure the latter was recorded as the surgeon However there appears (Table IV) to be very little difference if any in the incidence of complications between the three categories of physicians

## DISCUSSION

Along with the wide spread acceptance of liberal abortion laws and an ever increasing number of legal abortions publications on complication rates and sequelae in connection with VA have accumulated There are obvious differences between the reports as to the number of cases distribution by gestational week operation procedure definition of complications and extent of follow up The high complication rates in the present study differ from those of most other reports This may be due to

1 Unsatisfactory operative procedure and/or physician inexperience

2 Over representation of late first trimester terminations

3 Hospital admission for all patients and a long follow up period facilitating a careful recording of complications

It is evident that the proportion of late first trimester abortions in the present series was unusually high in that 75% of the patients were in the 11th week or later *A comparative evaluation of the total* complication rate is therefore misleading The results have to be compared in relation to gestational age and type of complication

The incidence of uterine perforation was 0 73% in the 11th and 12th week of pregnancy and 0 45% overall In current literature this type of complication is generally not reported as related to week of pregnancy However Tietze and Lewit (17) found a frequency of 0 2% in the 10th–11th week in their total case material and 0 5% in so called local patients with a more complete follow up In the 12th–13th week the corresponding figures were 0 3 and 0 4% respectively Stallworthy et al (15) recorded a perforation rate of 0 3% before the 10th week and as much as 2 5% after the 10th week

Nathanson (12) analysed the incidence of uterine perforation in 30000 legal abortions and found an overall frequency of 0 08% The author concluded that age parity uterine size or previous gynecological history were of no significance for the occurrence The only two factors which seemed to be related to this complication were uterine position and physician inexperience Although the present series is too small to allow any definite conclusions on this point it should be noted that 4 out of the 5 perforations occurred in primigravid patients

There was a high incidence of heavy uterine bleeding in the present series and the results differ significantly from the majority of other reports (6 7 8 11 16 17 20 21)

In the 10th week of pregnancy 2 8% of the cases had a blood loss exceeding 500 ml and the corresponding figure in the 11th week was 3 2% provided that halothane anaesthesia had not been utilized This incidence is higher than the 0 8% (total case material) and 1 1% (local patients with follow up) reported by Tietze and Lewit (17) It should however be emphasized that in the latter study *the amount of blood loss was usually not measured* only estimated and that the conceptus (fetus placenta and amniotic fluid) was included in the measured amount in the present study However in the 12th–13th week the two series differed significantly in that 16 1% (12th week) and 21 8% (≥13th week) had a blood loss of more than 500 ml whereas the corresponding figures in Tietze and Lewit s series were 2 1 and 2 8% (local patients) Apart from the latter study there are comparatively few publications where uterine haemorrhage is strictly recorded in relation to week of pregnancy Vojta (21) reported an incidence of 4 1% with a blood loss of 300 ml or more in the 9th–13th week as compared to nil in the 6th–8th week Fåhraeus (5) reported excessive uterine haemorrhage (500 ml or more) in 3 3% in the 12th week or later and 0 2% before the 12th week of gestation On the other hand in Stallworthy s et al (15) series there was *an estimated blood loss of 500 ml or more in* 21 4% of the patients after the 10th week and according to Loung et al (9) 27% in the 12th–14th week There was a significantly lower incidence of uterine bleeding at an earlier gestational age in both these latter reports Benc et al showed that the average measured and recorded blood loss during VA was 2–3 times larger in the 12th week than in the 7th–8th week of pregnancy (1)

The data of the present study refer to results obtained without halothane anaesthesia The situation is completely different in the series given halothane in that an unacceptable blood loss was sometimes recorded also in the early cases (Fig 2) In the 10th and 11th week there was a 5–6 fold increase in the incidence of bleeding illustrating a definite contraindication to the use of this anaesthetic in connection with VA However it should be mentioned that the study was retrospective which means that halothane in some cases might have been administered for a special reason In cases where the VA procedure met with technical difficulties the duration of the anaesthesia had to be prolonged necessitating supplementation with halothane

*These results indicate that there is a* definite relation between blood loss and week of pregnancy and also that a considerable variation exists between different publications The present study revealed an unacceptably high rate of excessive uterine haemorrhage in the 12th and 13th week of gestation

Late uterine bleeding requiring re-curettage occurred in 1 4% of the cases This figure is in accordance with the results of the majority of other reports (1 5 9 10 13 15 20 21)

Pelvic infection is a *heterogenous concept* that

is generally based both on subjective symptoms and crude objective signs Accordingly the definitions vary to a great extent from one author to another and consequently also the incidence that ranges between 3–9% (1 6 7 9 10 13 14 15 17 19 20) An early infection rate of 2 8% and a late infection rate of 4 4% found in the present case material seems high compared to most reports Whether this is due to inadequate technique or should be attributed to thorough supervision and follow up cannot be stated However according to Tietze and Lewit (17) hospital supervision of the post operative course definitely increases the rate of recorded infections

On the whole the total complication rate in the present study was high particularly the incidence of uterine haemorrhage We believe that this high incidence is mainly due to factors related to the technical performance of the abortion The comparatively low negative pressure used (0 4 instead of the generally recommended pressure of 0 7 kg/cm²) is inefficient for the achievement of a disconnection of the ovum from the uterine wall and an easy passage of the products of conception through the tubing which is a prerequisite for rapid evacuation of the uterine contents Administration of i v oxytocin (high rate infusion or high dose single injection) at the beginning of the operation initiates uterine contraction and reduction of the size of the cavity which is of great importance in preventing large uterine haemorrhage (6) It has been shown that halothane inhalation is associated with uterine relaxation and increased uterine haemorrhage (3 4) This form of anaesthesia should therefore be avoided The above mentioned criteria for the technical performance of VA have now been introduced in the routine of this department and this policy has definitely reduced the complication rate (to be published)

It seems reasonable to assume that physician in experience might have contributed to the comparatively bad results in view of the fact that the abortion operations were distributed among a large number of staff members In general the individual physician irrespective of training category did not carry out more than two–three abortions per week compared with 10–15 cases a day which is a common figure in many countries with a high incidence of first trimester abortion and a low complication rate (2) The present results indicate that an experienced gynecologist *per se* is no guarantee for a low complication rate unless he has had a thorough training in performing VA

However the figures emphasize significantly the relation between uterine haemorrhage and late gestational age as all patients were managed in the same institution and following the same technical principles The fact that in patient treatment and close follow up tend to increase the apparent morbidity not only as found in the present series but also as judged from other studies indicates that the complication rate in late first trimester abortions might have been underestimated and that VA should not be considered a totally simple and harmless procedure (16)

*Abortion in the late stages of the first trimester* represents a problem not only with regard to immediate complications but also with reference to remote sequelae The rapid traumatizing mechanical dilatation of the cervix constitutes in particular a questionable procedure It would be of advantage if the dilatation could be achieved slowly The laminaria tent may fulfil this requirement but involves introduction of a foreign body in the uterus and hence an increased infection risk The prostaglandins have recently been utilized to induce preoperative dilatation of the cervix in late first trimester abortions The dilatation is achieved by a more physiological mechanism namely uterine contractions A single extra amniotic injection of a long acting prostaglandin analogue (15(S) 15 methyl $PGF_{2\alpha}$) has been shown to induce sufficient cervical dilatation to allow a simple instrumental evacuation of the uterus (22) The incidence of uterine haemorrhage (>500 ml) following this pretreatment in late first trimester abortions was only 3% as compared with approximately 20% found in the present series (18)

ACKNOWLEDGEMENTS

This study was supported by a grant from the Swedish Medical Research Council (grant 17X 2019-08C) We are indebted to AB Astra Sodertalje Sweden for assistance with computer analysis of the data

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*Submitted for publication Oct 18 1973*

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*Submitted for publication Oct 24 1973*


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Acta Obstet Gynec Scand 54 129–134 1975

# ACID BASE AND ELECTROLYTE BALANCE IN INFANTS OF DIABETIC MOTHERS

*Vaginal Delivery versus Caesarean Section*

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The acid-base and electrolyte balance of 30 was studied at delivery and in their infants during 48 h 18 women were diabetics 10 of these were d vaginally ($DM_{vd}$) and 8 by elective caesarean $DM_{cs}$) 12 healthy women were vaginally delivered The infants of diabetic mothers (IDM) received fusion therapy *At birth* the $DM_{vd}$ and their infants had a more pronounced metabolic acidosis than and their babies ($IDM_{cs}$) The largest metabolic occurred however in the group of $HM_{vd}$ and nts ($IHM_{vd}$) *After birth* no significant differences tained in the acid-base and electrolyte balance $IDM_{vd}$ and $IDM_{cs}$ The plasma potassium level d lower in IDM than in IHM The study stresses the ce of adequate management of diabetes in preg n combination with active intravenous therapy elivery and to the infant in the immediate neonatal The slightly larger metabolic acidosis seen in com with vaginal delivery suggests that this mode of should not be attempted uncritically in diabetic

id base and electrolyte balance in infants of mothers (IDM) delivered by caesarean sec s previously been studied at birth and during t 48 hours after birth (13 15) At birth the se balance of IDM was similar to that of of healthy mothers (IHM) but in the early d period the IDM had slightly higher pH and lower levels of $P_{CO_2}$ and plasma potas an the IHM This difference was probably to the higher caloric intake in IDM caused by travenous fluid therapy and early feeding

develop a combination of respiratory and lic acidosis after birth which is more pro- d after vaginal delivery than after caesarean and it can be assumed that the mode of delivery affects the acid base balance of IDM in a similar way

The aim of the present investigation was to study the acid base and electrolyte balance in vaginally delivered IDM at birth and during the first 48 hours after birth The results obtained were related both to those of a group of IDM delivered by caesarean section and to those of a group vaginally delivered IHM

## MATERIAL AND METHODS

*Diabetic group*

Eighteen diabetic mothers were studied 10 were delivered vaginally ($DM_{vd}$) and 8 by elective caesarean section ($DM_{cs}$) The $DM_{cs}$ and their infants have been described earlier (15) but a short presentation of both groups is given below The maternal age was 21 to 31 years in $DM_{vd}$ and 22 to 38 years in $DM_{cs}$ The severity of the diabetes is indicated in the table below where the mothers are grouped according to White s classification (18)

| | A | B | C | D | F+R | Total |
|---|---|---|---|---|---|---|
| $DM_{vd}$ | 1 | 3 | 5 | 1 | – | 10 |
| $DM_{cs}$ | – | – | 4 | 3 | 1 | 8 |

Throughout pregnancy the diabetes was well controlled within the limits for blood sugars accepted in modern treatment of diabetic pregnancy (8 10)

During pregnancy 1 $DM_{vd}$ and 4 $DM_{cs}$ developed toxaemia 1 $DM_{vd}$ acute pyelitis and 1 $DM_{cs}$ hepatosis Another $DM_{vd}$ carried monozygotic twins one of which died before delivery (the autopsy showed aplasia of one umbilical artery and widespread infarction of the placenta)

The $DM_{cs}$ were delivered at 37 to 38 weeks of pregnancy ($\bar{x}$=37.5 weeks) and the $DM_{vd}$ at 37 to 40 weeks ($\bar{x}$=38 3 weeks) In all $DM_{vd}$ labour was induced by infusion of

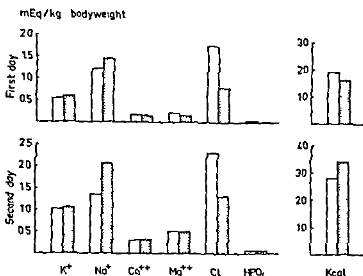



*Fig 1* Daily electrolyte (mEq/l bodyweight) and caloric intake (kcal) The hatched columns represent the values of $IDM_{vd}$ and the unfilled columns the values of $IDM_{cs}$

oxytocin in 5 5% glucose Vacuum extraction was used for delivery in 4 DM , in 1 due to preeclampsia and in 3 to prevent the second stage exceeding 60 minutes as has been recommended by Jacobson (6) in order to avoid the development of fetal acidosis

The birth weights ranged from 2 710 to 4 590 g ($\bar{x}$=3 498 g) in infants of vaginally delivered diabetic mothers ($IDM_{vd}$) and from 1 950 to 3 930 g ($\bar{x}$=3 110 g) in infants of diabetic mothers delivered by caesarean section (IDM ) Three of the 10 $IDM_{vd}$ and 1 of the 8 $IDM_{cs}$ had birth weights above the 90th percentile

*Control group*

The control group consisted of 12 healthy vaginally delivered mothers ($HM_{vd}$) and their infants ($IHM_{vd}$) These mothers aged 18 to 35 years with no complications during pregnancy were delivered spontaneously at 37 to 41 weeks of pregnancy ($\bar{x}$=40 weeks) Three vacuum extractions were performed in order to shorten the second stage Their abies weighed 2 500 to 4 000 g ($\bar{x}$=3 320 g) and none had a birth weight above the 90th percentile This group is identical with the control group in a recent study on epidural analgesia (16 17) The obstetric analgesia was similar in the two vaginally delivered groups

*Postnatal care*

The $IHM_{vd}$ were placed in incubators for close observation during two hours after birth at the Department of Obstetrics while all IDM were immediately transferred to the neonatal ward at the Department of Paediatrics and placed in incubators for two days

The IDM received intravenous fluid from the first hour after birth and onwards with a solution containing 5% glucose 5% fructose and electrolytes at a rate of 2 5 ml/kg/hour Early feeding was started 6 hours after birth The daily electrolyte and caloric intake was similar in both $IDM_{vd}$ and $IDM_{cs}$ (Fig 1) The discrepancy in sodium and chloride intake was due to repeated infusions of sodium bicarbonate to one vaginally delivered infant with hyaline membrane disease treated on a respirator The $IHM_{vd}$ were studied only during the two first hours after birth and received no intravenous fluid or feeding during this period

*Blood sampling and analytical methods*

Before the study informed consent was obtained from both parents At birth blood was taken by puncture from the umbilical artery and vein respectively At the same time a blood sample was taken from a peripheral vein of the mother without stasis In $IDM_{vd}$ and $IDM_{cs}$ an umbilical artery catheter was introduced and blood samples were drawn every hour during the first 4 hours and at 6 24 and 48 hours The sampling procedure and analytical methods have all been described in detail earlier (12 13 15) In $IHM_{vd}$ blood was obtained by femoral puncture at 1 and 2 hours

## RESULTS

*Clinical condition and behaviour of the newborn infants*

The condition at birth was satisfactory in all $IDM_{cs}$ in 7 $IDM_{vd}$ and in 11 $IHM_{vd}$ Two $IDM_{vd}$ had an initial Apgar score of 6 one $IHM_{vd}$ a score of 7 but these scores were normal at 10 minutes A third $IDM_{vd}$ developed hyaline membrane disease was treated with a respirator but died after 2 days

During the first two days after birth the following symptoms were recorded 6 $IDM_{vd}$ and 5 $IDM_{cs}$ were hyperexcitable 4 $IDM_{vd}$ and 3 $IDM_{cs}$ had a marked postnatal weight loss 2 $IDM_{vd}$ and 1 $IDM_{cs}$ had respiratory distress Hyperbilirubinaemia occurred in 6 $IDM_{vd}$ and in 1 $IDM_{cs}$ hypoglycaemia in 1 $IDM_{vd}$ and 1 $IDM_{cs}$ and polycythaemia in 4 $IDM_{vd}$ In IHM none of these symptoms were observed

*Effect of mode of delivery on DM and IDM*

*At birth (Table I Fig 4)* The acid base parameters in maternal blood showed a more pronounced metabolic acidosis and lower $P_{CO_2}$ values in the

Table I *The effect of the mode of delivery on different chemical constituents in maternal venous and umbilical arterial plasma*

Comparison between the values of the vaginally delivered DM DM delivered by caesarean section and their infants $x$=mean value

| Analysis | Maternal | | | Neonatal | | |
|---|---|---|---|---|---|---|
| | $x_{DM_{vag}}$ | $x_{DM_{cs}}$ | Significance of differences | $x_{IDM}$ | $x_{IDM_c}$ | Significance of differences |
| pH | 7 436 | 7 455 | – | 7 280 | 7 370 | |
| $P_{CO_2}$ | 23 1 | 27 0 | | 43 5 | 39 8 | – |
| $BD_{Hbs}$ | 8 2 | 5 5 | | 6 0 | 2.5 | |
| Potassium | 3 6 | 3 6 | – | 5 1 | 3 9 | – |
| Sodium | 13? 4 | 134 4 | – | 136 1 | 140 3 | – |
| Chloride | 104 5 | 106 1 | – | 106 0 | 109 6 | – |
| Bicarbonate | 14 9 | 18 2 | | 19 4 | 21 7 | – |
| Total calcium | 4 4? | 4 74 | – | 5 55 | 5 54 | – |
| Total protein | 17 8 | 15 9 | * | 13 9 | 13 1 | – |
| Inorganic phosphorus | 0 94 | 0 91 | – | 1 98 | 1 89 | – |
| Glucose | 1.4 0 | 141 5 | – | 9? 6 | 73 1 | – |
| Lactate | 4 0 | 1 9 | – | 4 3 | 2 0 | |

=0 05>$p$>0 01 ≈0 01>$p$>0 001 =0 001>$p$

DM $_g$ than in the DM while the pH did not differ between the two groups The mean metabolic acidosis in the 4 DM $_a$ delivered by vacuum extraction tended to be slightly higher than the mean for the whole group and was 9 3 mEq/l (range 7 9–11 4 mEq/l) in the mothers and 7 7 mEq/l (range 5 6–12 7 mEq/l) in their babies In umbilical arterial blood of IDM $_{ag}$ pH was below and $BD_{Hbs}$ above corresponding values of $IDM_{cs}$

The electrolyte glucose and lactate values were similar in DM $_g$ and DM A lower plasma bicarbonate was found in DM $_g$ and a higher level of total protein In umbilical arterial plasma no differences were seen between the electrolytes glucose and lactate of the two groups

*During the first 48 hours after birth (Figs 2 and 3)* The differences found at birth between the IDM $_g$ and the $IDM_{cs}$ quickly disappeared and during the next 48 hours the levels of all the chemical constituents determined in blood of IDM $_g$ tended to be similar to those of $IDM_{cs}$

### *Effect of maternal diabetes on acid base and electrolyte balance*

*At birth (Table II Fig 4)* The DM $_{ag}$ showed a lower degree of metabolic acidosis than the HM $_g$ The pH was higher in DM $_g$ than in HM $_g$ while the $P_{CO_2}$ levels did not differ The mean metabolic acidosis in the 3 HM $_g$ delivered by vacuum extraction tended to be slightly higher than the mean for the whole group and was 12 6 mEq/l (range 9 8–16 6 mEq/l) and 11 6 mEq/l (range 10 2–14 1 mEq/l) for the babies In IDM the umbilical arterial blood showed a lower degree of metabolic acidosis and a concomitant higher level of $P_{CO_2}$ than corresponding values in IHM $_g$ These changes resulted in similar pH values in both IDM $_g$ and IHM The plasma electrolytes were also similar in both groups

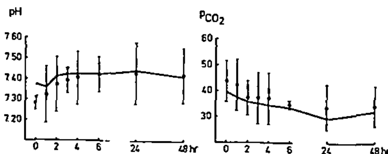



*Fig 2* pH and $P_{CO_2}$ in arterial blood of IDM $_{ag}$ during 48 hours after birth The mean values±2 standard deviations denoted by dots and vertical bars are plotted against corresponding values of $IDM_{cs}$ (The shaded area and the whole line in Figs 2 and 3 show the corresponding mean values±2 standard deviations for the 8 $IDM_{cs}$ )

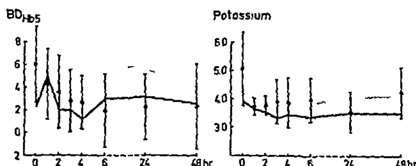



*Fig 3* $BD_{Hb5}$ and potassium in arterial blood of $IDM_{vag}$ during 48 hours after birth The mean values ±2 standard deviations are plotted against corresponding values of $IDM_{cs}$ ($BD_{Hb5}$ and potassium in mEq/l)

*During the first 2 hours after birth (Table II)* During the first 2 hours the level of metabolic acidosis was less pronounced in $IDM_{vag}$ than in $IHM_{vag}$ and during the same time period the levels of plasma potassium in $IDM_{vag}$ remained below those of $IHM_{vag}$

No differences were seen in the concentration of electrolytes glucose or lactate in maternal or neonatal blood of $IDM_{vag}$ and $IHM_{vag}$

## DISCUSSION

The optimal time for delivery of diabetic women is usually set at 36 to 37 weeks but lately the modern treatment of diabetic pregnancies has made it possible to postpone the time of delivery (8) As to the mode of delivery the opinions vary widely Caesarean section has always been most liberally used but lately the frequency of vaginal deliveries seems to be increasing It is claimed that in properly selected diabetic women with carefully managed labour the risk for the baby is not higher by this mode of delivery (8 10) Also if vaginal delivery is chosen the postoperative problems in the diabetic mother are avoided

In the present study both groups of IDM were delivered at a mean gestational age of about 38 weeks while the IHM were delivered at 40 weeks The vaginal delivery induced by oxytocin might be less advantageous than elective caesarean section as to the degree of metabolic acidosis which is somewhat higher in vaginal delivery The higher lactate values and lower $P_{CO_2}$ values found in the $DM_{vag}$ in the present study were probably the result of the muscular work and hyperventilation accompanying labour

The higher level of total protein and slightly lower glucose concentration in $DM_{vag}$ compared to $DM_{cs}$ might be dependent on a minor difference in the treatment In $DM_{cs}$ a solution of 5 5 per cent glucose was infused at a fairly high rate immediately before and during operation while the infusion in $DM_{vag}$ was performed at a lower rate but during the entire period of labour

The pH in umbilical arterial blood at birth was much lower in $IDM_{vag}$ than in $IDM_{cs}$ due to a com

Table II *The effect of maternal diabetes on different chemical constituents in maternal and neonatal blood*

Comparison between the values of the diabetic and the control group ($DM_{vag}$-$HM_{vag}$ and $IDM_{vag}$-$IHM_{vag}$) $x$=mean value Diff =difference Sign =significance

| | MV | | | UA | | | 1 hour | | | 2 hours | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $x_{DM\ vag}$ | Diff | Sign | $x_{IDM\ vag}$ | Diff | Sign | $x_{IDM\ vag}$ | Diff | Sign | $x_{IDM\ vag}$ | Diff | Sign |
| pH | 7 436 | 0 074 | * | 7 280 | 0 007 | – | 7 321 | 0 026 | – | 7 371 | 0 027 | – |
| $P_{CO_2}$ | 23 1 | 0 3 | – | 43 5 | 9 1 | * | 42 1 | 6 9 | – | 37 1 | 3 7 | – |
| $BD_{Hb5}$ | 8 2 | −3 5 | | 6 0 | −4 1 | | 4 2 | −4 5 | | 3 6 | −3 3 | |
| Potassium | 3 60 | −0 35 | – | 5 05 | 0 28 | – | 3 73 | −1 00 | * | 3 76 | −1 06 | |
| Sodium | 132 4 | −1 2 | – | 136 1 | 0 7 | – | 133 6 | −6 3 | – | 138 6 | −1 3 | – |
| Chloride | 104 5 | −2 0 | – | 106 0 | 2 0 | – | 106 3 | −0 9 | – | 108 5 | 3 3 | – |
| Glucose | 124 0 | 21 3 | – | 92 6 | −0 8 | – | 53 6 | −12 4 | – | 4[illegible] 6 | −12 8 | – |
| Lactate | 4 0 | 0 7 | – | 4 3 | 0 7 | – | 2 8 | −1 0 | – | 2 0 | −0 6 | – |

*=0 05>$p$>0 01 **=0 01>$p$>0 001 *** =0 001>$p$

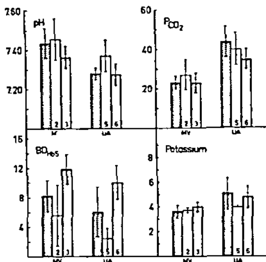



*Fig 4* Mean values and standard deviations of acid-base parameters and plasma potassium in maternal venous (MV) and umbilical arterial (UA) blood at birth The hatched columns represent the values of $DM_{vag}$ (1) and $IDM_{vag}$ (4) the unfilled columns the values of $DM_{cs}$ (2) and $IDM_{cs}$ (5) and the fine stippled columns illustrate the values of HM (3) and $IHM_{vag}$ (6) respectively

bined metabolic and respiratory acidosis in the former This acidosis disappeared soon after birth as one hour later the acid base parameters were within the limits for those of $IDM_{cs}$ This tendency towards metabolic acidosis in the $IDM_{vag}$ and in their mothers was seen in spite of the fact that the $DM_{vag}$ were in a more favourable situation regarding their diabetes than the $DM_c$ Only women with a not too long duration of diabetes and with good sugar control were considered candidates for vaginal deliveries

The $IHM_{vag}$ showed a more pronounced decrease in pH during and after delivery than both $IDM_{vag}$ and $IDM_{cs}$ This might be due both to maternal and neonatal factors The DM were strictly controlled during pregnancy and had normal or almost normal glucose levels during the last month before delivery They were also closely supervised during the delivery and received a continuous glucose infusion during labour or operation The IDM received an intravenous infusion with glucose and fructose starting within 1 hour after birth

Authors disagree about the effect on the infant s acid base balance at birth of administration of glucose to women in labour Romney and Gabel (11) administered one dose of 25 g glucose i v followed by infusion of 15 g glucose per hour and reported a positive effect on the acid base status in umbilical arterial and venous blood but also a maternal glucose level of 220 mg/100 ml Anderson et al (1) on the other hand observed no effect on the acid base balance after a single i v dose of 60 g glucose

Immediate postnatal intravenous fluid therapy with glucose or glucose fructose and early feeding of IDM has been used by several authors (4 5 7) in order to prevent hypoglycaemia acidosis and hyperbilirubinaemia In the present study a glucose fructose solution with added electrolytes was given 1 hour after birth and early feeding started about 5 hours later if the baby was well No unphysiological variations in lactate levels occurred during the infusion of glucose fructose and the electrolyte intake was small enough to be readily excreated in the urine (9)

The levels of sodium chloride total calcium and inorganic phosphorus did not differ between the three groups at birth or in the infants afterwards They varied similarly in both IDM and IHM and also in vaginally delivered babies and caesarean section babies of the diabetic mothers The level of potassium in umbilical arterial plasma always somewhat higher than corresponding maternal levels tended to be higher in IHM and IDM delivered vaginally than in IDM delivered by caesarean section and could reflect minor interference in the umbilical circulation (2 14) Except for this initially high level of plasma potassium in $IDM_{vag}$ the potassium levels remained fairly stable during the first two days after birth and did not differ between the two groups of IDM The potassium levels in $IHM_{vag}$ were about 1 0 mEq/l above those in $IDM_{vag}$ throughout the first two days This difference could be related to an increased glucose uptake during the continuous glucose fructose infusion to the $IDM_{vag}$ with resulting deposition of glycogen and potassium due to an increased insulin output (2)

The results of the present study do not answer the question as to which mode of delivery is most suitable for diabetic mothers and their babies caesarean section or vaginal delivery The slightly more pronounced changes in acid base balance and in potassium levels of the $IDM_{vag}$ however in spite of the better conditions of their mothers might be taken as a sign that vaginal delivery should not be attempted uncritically in diabetic women The over all good control of blood chemistry recorded in the infants of the diabetic group also confirms the benefits both of adequate blood sugar control in the

mothers and of active therapy during the delivery and to the infants in the early neonatal period

## ACKNOWLEDGEMENTS

The authors wish to thank Mrs Inger Unnerstad for expert laboratory assistance and for drawing the figures This study was supported by a grant from Svenska Diabetes stiftelsen

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Submitted for publication Nov 9 1973

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Acta Obstet Gynec Scand 54 135–139 1975

# SERUM LEVELS OF OESTRADIOL AND PROGESTERONE DURING ADMINISTRATION OF PROSTAGLANDIN $F_2\alpha$ FOR INDUCTION OF ABORTION AND LABOUR

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*Abstract* Prostaglandin $F_2\alpha$ was used to induce abortion or labour in 84 women between the 11th and 44th weeks of pregnancy Three different routes of administration were used intravenous extra amniotic and intra amniotic The extra amniotic infusion of prostaglandin $F_2\alpha$ produced a faster response than the other two routes Less than one third of the dose used in the intra amniotic group was required in the extra amniotic group for a complete evacuation of the uterus Vomiting and diarrhoea occurred in 40% of the women in the intra and extra amniotic group while the frequency was 88% in the intravenous group Serum levels of progesterone and oestradiol decreased in accordance with the pattern found during spontaneous deliveries The effect of prostaglandin $F_2\alpha$ on the myometrium does not appear to be mediated via changes in the blood levels of progesterone or oestradiol

The use of prostaglandins to induce abortion or labour has been widely studied The mechanism by which prostaglandins produce uterine contractions remains unclear Possible mechanisms of different kinds have been suggested Csapo (6) Bengtsson et al (2) indicated that myometrial stimulation is mediated by removal of a progesterone block and that the myometrial response could result solely from a suppression of placental function Csapo et al (7) and Csapo (8) even demonstrated a continued decrease in oestradiol 17$\beta$ and serum progesterone during the intravenous administration of PG$F_2\alpha$ for inducing abortion Speroff et al (21) did not find significant hormonal changes prior to abortion but there was a clear decline in plasma oestradiol and oestriol levels during the intravenous infusion of PG$F_2\alpha$ The reports of Wiquist et al (24) and Hillier et al (14) confirm the unchanged level of progesterone during the PG$F_2\alpha$ infusion Wentz et al (23) have suggested that prostaglandins appear to exert effects on placental steroidogenesis primarily related to vascular impairment and anoxia but a direct effect on steroidogenic pathways may exist An oxytocic like stimulation of the myometrium has been suggested by Bygdeman et al (4) According to Anderson et al (1) the maternal plasma oestriol levels in late pregnancy are suppressed during induction of labour with PG$F_2\alpha$ but not with oxytocin

It has been shown in vitro that human myometrial strips exhibit an enhanced response to oxytocin after exposure to the $E_2$ but not $F_2\alpha$ prostaglandins (11) Uterine contractions can also be enhanced in vivo if oxytocin is given after exposure to PG$F_2$ (20) Stimulation of oxytocin release by infused PG$E_2$ and $F_2\alpha$ has been described recently (1?) The aim of our study was to investigate the serum oestradiol and progesterone levels during prostaglandin infusions

## MATERIAL AND METHODS

Prostaglandin $F_2\alpha$ was used to induce abortion or labour in 84 women between the 11th and 44th weeks of pregnancy The women were healthy and the pregnancies had all developed normally Thirty nine of the women were nulliparous while 45 had been pregnant before Prostaglandin $F_2\alpha$ was given by three different routes intravenous extra amniotic or intra amniotic (Table I)

The intravenous infusion was given through an antecubital vein by means of an infusion pump to ensure constant infusion The PG$F_2\alpha$ solution contained 15 $\mu g$/ml for induction of labour and ?94 $\mu g$/ml for induction of abortion

The extra amniotic infusion was given through a Foley catheter No 14 introduced through the cervical canal

Table I *Dose and route of administration of prostaglandin $F_{2\alpha}$*

The number of patients in each group is given together with the mean dose required and the average time and range until abortion or delivery

| Gestation week | | Extra amniotic | Intra amniotic | Intravenous |
|---|---|---|---|---|
| 11–14 | Patients | 10 | 4 | – |
| | Mean dose mg | 5 8 | 20 0 | – |
| | Time hours | 16 2 (2 1–16 5) | 10 6 | – |
| 15–19 | Patients | 27 | 5 | 3 |
| | Mean dose mg | 9 5 | 45 0 | 36 8 |
| | Time hours | 16 2 (2 5–56 0) | 32 2 | 11 2 (?4 1–113 6) |
| 20–29 | Patients | 4 | 2 | 1 |
| | Mean dose mg | 17 8 | 62.5 | 91 4 |
| | Time hours | 28 1 (4 5–86.5) | 38 6 | 29 8 |
| 36–44 | Patients | – | – | 25 |
| | Mean dose mg | – | – | 2 1 |
| | Time hours | | | 8 4 (0 8–9 6) |

and placed between the uterine wall and the amniotic sac Some 250–750 µg of $PGF_{2\alpha}$ was injected once or twice hourly by this route In the intra amniotic group $PGF_{2\alpha}$ was administered as a single dose 25 mg trans abdominally and repeated after 24 hours if necessary

Three venous blood samples were taken from each patient (1) immediately before the administration of $PGF_{2\alpha}$ (2) at the peak of myometrial contractions prior to the expulsion of the fetus and (3) about 24 hours after the abortion or the delivery The blood samples were trifuged and the serum was removed and stored in a ep-freeze at −20°C until assayed

Progesterone in serum was assayed by a competitive protein binding technique (16) Oestradiol in plasma was assayed by a radioimmunoassay (10)

## RESULTS

On average 2 1 mg of $PGF_{2\alpha}$ was required to induce labour from the 33rd to the 44th weeks of pregnancy The dose needed to induce abortion was much higher The extra amniotic administration required on average a lower amount of

Table II *Serum levels of progesterone and oestradiol before treatment (I) at expulsion of the fetus (II) and 24 hours later (III) with number of samples in each period of pregnancy and the mean level*

| Progesterone Gestation week | No | X | Oestradiol Gestation week | No | X |
|---|---|---|---|---|---|
| *I Sample* | | | *I Sample* | | |
| 10–14 | 13 | 29 74 | 0–14 | 13 | 4 69 |
| 15–19 | 3 | 30 37 | 15–19 | 34 | 4 86 |
| 20–29 | 7 | 38 81 | 20–30 | 7 | 9 77 |
| 36–44 | 26 | 171 30 | 35–44 | 24 | 24 88 |
| *II Sample* | | | *II Sample* | | |
| 10–14 | 31 | 11 46 | 0–14 | 11 | 1 68 |
| 15–19 | 32 | 14 11 | 15–19 | 3_ | 2 63 |
| 20–29 | 5 | 30 60 | 0–30 | 5 | 5 57 |
| 36–44 | 16 | 89 70 | 35–44 | 16 | _5 18 |
| *III Sample* | | | *III Sample* | | |
| 10–14 | 11 | 1 59 | 0–14 | 10 | 0 70 |
| 15–19 | 35 | 1 39 | 15–19 | 35 | 0 95 |
| 20–29 | 6 | 1 92 | 0–30 | 6 | 1 45 |
| 36–44 | 21 | 3 20 | 35–44 | 21 | 0 30 |

Table III *Statistically significant changes between the three different samples (Table II) in each period of pregnancy studied*

| Progesterone | | | Oestradiol | | |
|---|---|---|---|---|---|
| Gestation week | *t* | *p* | Gestation week | *t* | *p* |
| 1→2 | | | | | |
| 10–14 | 3 98 | <0 01 | 10–14 | 1 97 | <0 1 |
| 15–19 | 5 60 | <0 001 | 15–19 | 2 89 | <0 01 |
| 20–29 | 0 48 | NS | 20–30 | 1 65 | <0 01 |
| 35–44 | 2 45 | <0 05 | 35–44 | 1 16 | <0 01 |
| 1→3 | | | | | |
| 10–14 | 7 67 | <0 001 | 0–14 | 3 07 | <0 05 |
| 15–19 | 14 37 | <0 001 | 15–19 | 7 81 | <0 001 |
| 20–29 | 4 15 | <0 01 | 20–30 | 3.27 | <0 05 |
| 35–44 | 6 56 | <0 001 | 35–44 | 10 14 | <0 001 |
| →3 | | | | | |
| 10–14 | 5 30 | <0 001 | | | |
| 15–19 | 8 31 | <0 001 | | | |
| 20–29 | 1.59 | <0 001 | | | |
| 35–44 | 6 40 | <0 001 | | | |

prostaglandins and decreased the time required for abortion as compared with the intravenous or intra amniotic routes (Table I) For the induction of abortion the intra amniotic injection required 3–4 times larger amounts of prostaglandins in compari son with the extra amniotic injection In general *the induction abortion time was shorter in the* extra amniotic group compared with the intra am niotic cases However large individual variations were seen Intravenous infusion was only used in four women in the 15th to 29th week of pregnancy

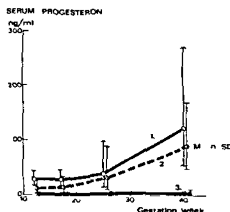



*Fig 1* Serum levels of progesterone during the four pe riods of pregnancy studied (Table I) 1 before treatment (O—O) 2 at the expulsion of the fetus ( ) 3 about 4 hours after abortion or delivery (●—●)

The mean time from induction to the complete abortion was 15 8 hours

The frequency of side effects such as vomiting and diarrhoea was 40% in the women to whom PG was given intra or extra amniotically However in the intravenous group the frequency was as high as 88%

The serum levels of progesterone and oestradiol during the experiment in the different groups are summarized in Table II The statistical significance of the changes seen between the preinfusion levels levels at the time of abortion or delivery and 24 hours after the expulsion of the fetus are shown in Table III Generally the progesterone levels de creased faster (Fig 1) during the administration than the oestradiol levels (Fig 2) The smallest dif ference was found in the 11–14 weeks of pregnancy for both progesterone and oestradiol In the 35–44 weeks of pregnancy the significance was somewhat smaller due to the large variation found between patients However when paired analyses were per formed the significance was very high

## DISCUSSION

The dose and route of prostaglandin administration during various periods in pregnancy reported in this paper demonstrate the well known fact that prostaglandin $F_2\alpha$ is a potent drug for the induc

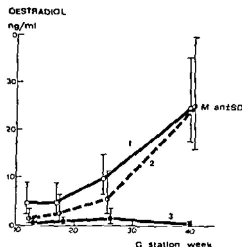



*Fig 2* Serum levels of oestradiol as in Fig 1

tion of abortion or labour The dose required varies with the route of administration From this study the extra amniotic route appears preferable as a lower dosage could be used thus minimizing the general side effects produced by high levels of prostaglandins in the peripheral circulation Synthetic prostaglandins appear to decrease the side effects still further (26)

The serum levels of progesterone and oestradiol were found to decrease in the same manner found during normal parturition (18) This finding agrees with recent publications with orally administered taglandin $E_2$ at term (9)

ring the early part of pregnancy there is still ersy as to whether the change in the plasma levels of progesterone and oestrogens precede myometrial contractions or vice versa (8 21) There was particular argument about the mode of action of hypertonic saline in inducing abortion (23) It now appears that the decreased levels of progesterone found before the expulsion of the fetus are not significantly correlated with the onset of clinical labour (17) However the onset of clinical labour is difficult to define which gives room for the opinion that the decreased levels of progesterone found before the expulsion of the fetus in saline induced abortions are essential for the onset of myometrial contractions (23)

It is interesting to notice that the hormonal changes were less pronounced in the period between the 10th and 14th weeks compared with the second half of pregnancy During this early period the corpus luteum is still functional and contributes to the plasma levels of progesterone as well as oestradiol even if the contribution decreases rapidly after the 10th week (15) In contrast to the corpus luteum of several lower animals the human corpus luteum is not affected by prostaglandins (5)

From several pieces of indirect evidence it would appear that prostaglandins are involved in the initiation of spontaneous labour and may even be concerned with normal menstruation through release of lysosomal enzymes which will stimulate prostaglandin synthesis and release An excellent review on this subject has recently been published by Gustavii (13) According to his theory the somewhat better effect produced by extra amniotic injection of hypertonic saline is explained by a more rapid release of prostaglandins In this connection it is interesting to note that the extra amniotic infusion of prostaglandin $F_2\alpha$ was the most effective route of administration

To sum up—it was found that the extra amniotic infusion of the prostaglandin $F_2\alpha$ was superior to the other routes studied The decrease of the serum levels of progesterone and oestradiol during and after the infusion of $PGF_2\alpha$ was not different from that seen during normal labour or spontaneous abortion (26) The action of prostaglandin $F_2\alpha$ on the myometrium does not appear to be mediated through changes in the progesterone or oestradiol levels

## ACKNOWLEDGMENTS

We wish to thank the Upjohn Company for supplies of prostaglandins

This study was supported by a grant from the Sigrid Jusélius Stiftelse Helsinki and by the Swedish Medical Research Council

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*Submitted for publication November 11 1973*

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Acta Obstet Gynec Scand 54 141–151 1975

# OUTCOME OF TERM BREECH DELIVERY IN PRIMIGRAVIDAE A FETO PELVIC BREECH INDEX

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*Abstract* Analysis of 340 term breech presentations in primigravidas showed a corrected perinatal mortality of 1 5% the elective cesarean section rate was 15% The incidence of complicated labour defined in the study was analyzed with regard to different parameters e g X ray pelvimetry data in all 340 cases Complicated labour in vaginal deliveries markedly increased with increasing fetal weight ($p<0\ 001$) and decreasing pelvic capacity ($p<0\ 001$) In each case the fetal weight and smallest pelvimetry data were given score points and the sum of these was called the Feto Pelvic Breech Index which was correlated to the incidence of complicated labour By using this index the mortality and morbidity probably can be markedly reduced without the routine use of elective cesarean section The prognostic methods available to detect feto-pelvic disproportion are discussed

It is a well documented fact that breech presentation is associated with increased fetal mortality and morbidity compared to vertex presentation This increased risk is partially due to the high incidence of prematurity The perinatal mortality after correction for antepartum deaths malformations and maternal complications in term (over 2 500 g) breech delivery is reported variously between less than 1% to around 5% The incidence of fetal injuries also shows considerable variation depending to some extent upon the type of injuries included in different studies

The long term prognosis of term breech infants delivered vaginally is not so well documented Among infants with cerebral palsy the incidence of breech delivery is reported to be about 9% (13 40) This is three times the general incidence of breech delivery but it should be pointed out that in about one third of the cases the infants were premature In the Collaborative Study of Cerebral Palsy there was no difference between breech and vertex presentation in 8 months motor and at one year neurologic development in infants with a birth weight over 2 500 g (3)

Analysis of the management of breech delivery in regard to the incidence of mortality and morbidity has not definitely clarified the preferable mode of delivery A more widespread use of cesarean section is recommended in several studies (e g 14 29 35 36) Wright (45) even advocates the routine use of cesarean section Prophylactic external cephalic version is proposed by some authors (4 15 21 26) According to Beischer & Townsend (1) the risk to the fetus in breech delivery is greater than the risk of version and subsequent cephalic delivery Most obstetricians seem to select cases with a high risk to the fetus for cesarean section resulting in a section rate between 5 and 25% in term breech presentation

When a vertex delivery takes place one risk to the fetus stems from mechanical feto-pelvic disproportion In term breech delivery there are also other problems such as the unmoulded head extended arms and increased incidence of fetal cord prolapse (11 14 17 42)

In numerous studies dealing with mortality and morbidity in breech delivery only a few authors have correlated outcome in vaginal delivery to the pelvic diameters obtained by roentgenography (e g Dunn et al (11) Johnson (19) Pearson (30) Perlmann (31) Todd & Steer (42))

The risk of an unsatisfactory outcome is even more pronounced when reduced pelvic capacity is associated with a large infant as has been pointed out recently by e g Rovinsky et al (35) However the risk to the fetus when these factors are

considered together has not previously been thoroughly documented

It is desirable to select those patients who probably should be delivered by cesarean section A *prognostic index for vaginal delivery in breech* presentation at term has been developed by Zatuchni & Andros (46 47) However although fetal weight parity gestational age and status determined by vaginal examination on admission to hospital are included pelvic capacity is not The main purpose of the present study was to analyse the outcome in regard to pelvic capacity especially with regard to the diameters of the pelvic outlet which hitherto have only been briefly considered (30 31) A contributory reason in this respect was the fact that the frequency of contraction of the pelvic outlet in Sweden is greater than that of inlet contraction as there was a sharp decline in the number of flat pelves after 1947 almost certainly due to antirachitic prophylaxis (5) Furthermore if possible a prognostic index for term breech presentation made from pelvimetry data combined with estimated weight or size of the skull of the fetus should be developed

## MATERIAL AND METHODS

The material was collected for two periods the first covering the period Sep 1 1962 to Aug 31 1966 at the Allmänna Barnbördshuset in Stockholm and the 2nd from Sep 1 1966 to Aug 31 1971 at the Allmänna Barnbördshuset and the Karolinska sjukhuset in Stockholm Breech presentation in primigravidas has for a long time been regarded as an indication for X ray pelvimetry at both these hospitals

The roentgenographic method used was that introduced by Borell & Fernstrom (5) and modified by Borell & Råberg (6) and Diehl & Fernstrom (8) With a small radiation dose this method gives information of high accuracy for both the inlet and outlet of the pelvis

The radiographs in this method are the following

1 A lateral view of the pelvis in the erect position for measurement of the sagittal diameter of the inlet (the shortest distance between the sacrum and upper dorsal border of the symphysis) and the sagittal diameter of the outlet (the distance between the sacral tip and lower dorsal border of the symphysis)

2 An antero-posterior view with an orthodiagraphic technique In this radiograph the widest transverse inlet the bispinous and bituberous distances can be measured directly

If the sagittal diameter of the inlet is below 10 cm contraction is considered to exist and disproportion often occurs so that cesarean section is performed in most cases When the sagittal inlet diameter is between 10 and 11 cm the sum of the distances of sagittal and transverse inlet should be at least 23 cm if the sum is smaller a so-called borderline pelvic inlet exists Contraction of the pelvic outlet is present when the sum of sagittal outlet bispinous and bituberous distances is below 29 5 cm A borderline pelvic outlet is present when the sum of the three diameters is between 29.5 and 31 5 cm

In vertex presentation the borderline has been based upon the outcome in 381 deliveries with small pelvimetric measurements selected from over 3 000 X ray pelvimetries (5) The numerical criteria of the borderline for vertex presentation have also been used for breech presentation In practice cesarean section was performed more often for breech than for vertex presentation in borderline cases

All pelvimetries with single breech presentation were primarily included in all 440 patients All X ray films were reexamined and measured excepting those with good diameters (sagittal inlet of 12 cm or more a transverse inlet above 12 cm and a sum of pelvimetric measurements of the outlet of 33 5 cm or more) All clinical records were studied except 8 which could not be found but there was indirect evidence of an uncomplicated delivery in 3 cases and in all 8 cases the pelvimetry data were normal These 8 cases were excluded and so also were 30 cases where cephalic version occurred spontaneously 31 cases where the patient was a multigravida 26 cases where the child was premature (<2 500 g) 4 children with malformations which could have influenced the results and one uncomplicated delivery at home

The corrected material then consisted of 340 term breech presentations in primigravidas

The following factors were recorded The age of the mother symptoms and medicaments during pregnancy duration of pregnancy all pelvimetric data mode and duration of delivery and complications as e g cord prolapse extended arms uterine inertia oxytocin stimulation signs of asphyxia and bleeding In all vaginal deliveries there was a report of the last stage of delivery from which the delivery was classified into 3 groups namely normal semidifficult and difficult Furthermore sex weight head circumference Apgar score and resuscitation of the infant were recorded Complicated cases were analyzed in detail and followed up in the records at the respective paediatric clinic

The pelvimetric data were analyzed as follows The sagittal and transverse diameters of the pelvic inlet were analyzed both separately and together with regard to the outcome in all 340 term breech presentations The separate measurements of the outlet and the sum of these measurements were also analyzed with regard to outcome in all deliveries In vertex vaginal delivery the risk of mortality and morbidity is statistically defined to be the same in contracted or borderline pelvic inlet as in contracted or borderline outlet In vertex labour contraction of the pelvic inlet is of course easier to detect clinically and to manage than contraction of the outlet In breech delivery the size of the inlet and that of the outlet are considered to be of the same mechanical importance In the present study

contraction of the inlet and outlet were therefore analyzed together and so also were borderline measurements of inlet and outlet

## RESULTS

### *Mode of delivery*

Table I shows the mode of delivery The total cesarean section rate was 17% with a higher incidence at the Karolinska sjukhuset (21 5%) than at the Allmanna Barnbordshuset (13 5%) In assisted delivery the Veit Smellie Mauriceau manoeuvre is the routine method in an uncomplicated case in both hospitals Pudendal anaesthesia was used in almost every delivery In no assisted labour were forceps applied to the after-coming head

The indication for cesarean section was contracted pelvis (diagnosed radiologically see Methods) in 34 cases which was relative (borderline) in 30 cases In 12 of these additional indications existed In all cases of relative contraction the fact that the patient was a primigravida with breech presentation was a more or less contributory indication The indication for emergency cesarean section was threatening fetal asphyxia in 7 cases which in one case was combined with a borderline pelvis in another with secondary uterine inertia and in a third with prolapse of the fetal cord In the remaining 18 cases of cesarean section other indications often multiple were present

The indication for extraction was threatening asphyxia in 5 cases and secondary uterine inertia in 2 cases Birth weight in 2 cases was below average and in both of these cord prolapse was the reason for the extraction In the other 5 cases

Table I

| Mode of delivery | No of cases | % | Mortality (corrected) % |
|---|---|---|---|
| Cesarean section (elective) | 52 | 15 | 0 |
| Vaginal delivery (primary) | 288 | | 1 7 |
| emergency cesarean section | 7 | 2 | (0/7) |
| extraction | 7 | 2 | (1/7) |
| assisted (or spontaneous) | 274 | 81 | (4/ 74) |
| Total | 340 | 100 | 1 5 |

Table II

| Mortality and morbidity | No of labours (n=288 cases) |
|---|---|
| Deaths | |
| Rupture of tentorium | 2 |
| Asphyxia | 3 |
| Injuries | |
| Brachial plexus paralysis (+ minor fracture) | 8 |
| Major fracture | 2 |
| Cerebral palsy and brachial paralysis | 1 |
| Lesion of the brain | 3* |
| Suspected injuries | |
| Brachial plexus paralysis (+ minor fracture) | 3 |
| Cerebral palsy | 1 |
| Paresis of the phrenic nerve | 1 |
| Total | 24 |

Two cases fetal cord complication
* Three cases fetal cord complication

the birth weight was over 3 500 g and in 3 of them over 4 000 g

### *Mortality*

The corrected perinatal mortality was 1 5% (5 cases) (Table I) In 2 of these 5 cases death was assigned to fetal cord complication In the remaining 3 cases death was possibly directly related to feto pelvic disproportion In one of these there was a rather big fetus (3 700 g) in combination with borderline measurements of the outlet in the second one the pelvis was normal but the infant weighed 4 240 g and both arms were extended and in the third case the birth weight was 3 300 g but the head was relatively larger with a circumference of 36 5 cm and this was considered to result in disproportion in combination with a pelvic inlet and outlet just over the upper limit of the borderline and full extension of both arms

### *Morbidity*

After exclusion of minor fractures without other symptoms fetal injuries (clear or suspected) occurred in 16 cases (6%) of primary vaginal deliveries after correction for 3 cases with fetal cord complications (Table II) No fetal injury occurred in elective cesarean section

At follow up examinations of infants with symptoms of brachial plexus injury at birth all were symptomfree within the first year A child with a

considered together has not previously been thoroughly documented

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If the sagittal diameter of the inlet is below 10 cm contraction is considered to exist and disproportion often occurs so that cesarean section is performed in most cases When the sagittal inlet diameter is between 10 and 11 cm the sum of the distances of sagittal and transverse inlet should be at least 23 cm if the sum is smaller a so called borderline pelvic inlet exists *Contraction of the pelvic outlet is present when the sum* of sagittal outlet bispinous and bituberous distances is below 29 5 cm A borderline pelvic outlet is *present* when the sum of the three diameters is between 29 5 and 31 5 cm

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Table VI

| X ray pelvimetry (smallest of inlet or outlet) | | Cases | Complicated labour | |
|---|---|---|---|---|
| Sag inlet (cm) | Outlet (sum of) | n (283) | n (39) | % |
| ≥12 0 | ≥33.5 | 144 | 13 | 9 |
| 11 5–11 9 | 32 5–33 4 | 73 | 12 | 16 |
| 11 0–11 4 | 31 5–32 4 | 46 | 5 | 11 |
| ≤10 9 | ≤31 4 | 20 | 9 | 45 |

tion to pelvic capacity increased markedly from the normal to the borderline group ($p<0\ 001$) Complicated labour indicating feto-pelvic disproportion occurred in as many as 9 out of 20 (45%) borderline cases (Table VI) The only vaginal delivery with a contraction of pelvis was not defined as complicated and the sagittal diameter of the inlet was 9 8 cm (Table VII) However the infant weighed only 2970 g and had a head circumference of 33 cm At birth 27 days prior to term the Apgar score was only 6 points after 5 minutes but the infant recovered steadily without any sequelae

Very complicated labour increased with decreasing pelvic capacity The correlation between pelvic capacity and very complicated labour statistically could only be divided into two groups There was a small increase in the incidence of very complicated labour in the group with a sagittal inlet diameter smaller than 11 5 cm or a sum of outlet measurements smaller than 32 5 ($p<0\ 025$)

In the present series of 340 primigravidas a contracted pelvis was found in 5 cases and a border line pelvis in 51 other cases totalling 16 5% (Table VII) This incidence is somewhat smaller than in 1 160 consecutive pelvimetries examined during a period of 4 years (1966–1969) at the Karolinska sjukhuset In this series contraction was *found in 2 7% and a borderline inlet or outlet in* 18% (Ohlsen unpublished data) In the present study contracted or borderline pelvic outlet was more common than diminution of the inlet

6 *Combination of birth weight and X ray pelvimetry* (Fig 1) The main factors in feto-pelvic disproportion are the size of the infant and the pelvic capacity Statistically these two independent factors showed about the same significant $\chi^2$ values with regard to complicated labour Therefore they ought to be considered equally important when analyzed in combination According to Fig 1 increasing birth weights were given increasing score points (from 1 to 4 points) and the smallest pelvimetry data of either the sagittal diameter of the inlet or the sum of the outlet measurements were in the same manner given score points (from 1 to 4 points) The combination of the two factors fetal weight (F) and the smallest pelvic capacity (P) was then calculated from the sum of score points of these two factors in each case The incidence of complicated labour was as expected highly significantly increased ($p<0\ 0005$) when a small pelvis was present together with a large infant i e with an increasing sum of score points (F+P) The sum of score points was called Feto Pelvic Breech Index

7 *Elderly primigravidae* No increased incidence of complicated labour was found in 32 pa

*Table VII*

| Pelvic capacity (Vertex presentation See Methods) | Breech n (340) | Cesarean section (elective) n (52) | Vaginal delivery (primary) Complicated n (44) | Uncomplicated n (244) |
|---|---|---|---|---|
| Contraction of | | | | |
| inlet | 2 | 1 | – | 1 |
| outlet | 3 | 3 | – | – |
| inlet & outlet | – | – | – | – |
| Borderline of | | | | |
| inlet | 16 | 7 | 4 | 5 |
| outlet | 28 | 17 | 6 | 5 |
| inlet & outlet | 7 | 6 | 1 | – |
| Normal | 284 | 18 | 33 | 233 |

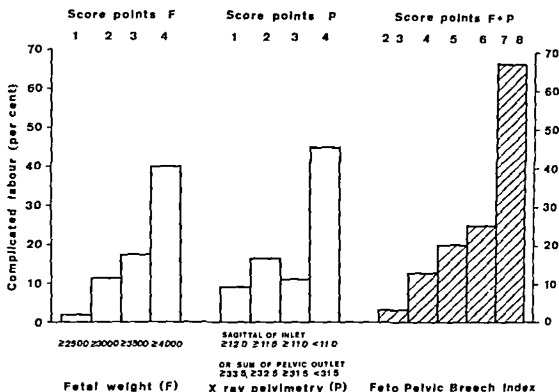



*Fig 1* Complicated labour in per cent with regard to fetal weight and smallest pelvic capacity in 283 breech deliveries In each case score points can be calculated from estimated fetal weight (F) and smallest pelvic capacity (P) The sum of these (F+P) gives the score point of Feto Pelvic Breech Index For a certain index the prognostic incidence of a complicated labour can be seen

tients over 30 years of age and there was no primigravida over 35 years of age in 39 cases of complicated labour

## DISCUSSION

### *Material*

The possibility that some primigravida with a term breech presentation did not have X ray pelvimetry is very low since breech presentation is a well known indication in both hospitals If such was the case and if any complication occurred pelvimetry was performed within a few days after delivery

In a few cases in the present corrected material slight toxaemia Rh immunization or some other factor might jeopardize the condition of the fetus but in none of the cases with complicated labour was there any reason to suspect any significant influence from such factors

Table VIII

| Author | No of breech | Primi gravidae | Multi gravidae | Fetal weight (g) 2 500–2 999 | 3 000–3 499 | 1 500–3 999 | 4 000– |
|---|---|---|---|---|---|---|---|
| Hall et al (18) | 4 515 | + | + | 34 | 42 | 19 | 5 |
| Dunn et al (11) | 187 | + | | 32 | 38 | 25 | 9 |
| Johnson (19) | 199 | + | | 27 | 40 | ?6 | 7 |
| Bird & McElin (2) | 500 | + | + | 34 | 38 | 21 | 6 |
| Rovinsky et al (35) | 2 145 | + | + | 34 | 4? | 19 | 5 |
| Own material | 340 | + | | 2? | 40 | 31 | 7 |

### *Mode of delivery*

The cesarean section rate of 17% is somewhat smaller than other reported incidences of 23–30% (1? 14 42) and the incidence of 20 4% in primigravidas picked out from a large series studied by Hall et al (18) On the other hand the incidence in the present series is higher than that in primigravidas reported by Bird & McElin (2) (11%) and Johnson (19) (13%)

The frequency of 2% for extraction in the present series is significantly lower than in all studies known to the author (e g 7 9 10 35 38)

### *Mortality and Morbidity*

The corrected perinatal mortality of 1 5% in the present study is in good agreement with the average figures reported by most other authors In some series where the same corrections can be made as in the present one the mortality in series comprising more than one hundred primigravidas with term vaginal breech delivery vary from 0 6% to about 6% (11 19 29 38)

Conditions regarded as fetal injuries vary between different authors according to different opinions and with the information given in the records For instance should isolated fracture of the clavicle diagnosed with or without X ray be included? Rubin & Grimm (36) found injuries in 6 7% infants half consisted of central nervous system or brachial plexus injuries Zatuchni & Andros (47) reported 5 injuries in 139 cases and Bird & McElin (2) only 9 injuries in 500 deliveries and all these 9 cases had only minor fractures Johnson (19) found injuries including different types of fractures in 10% of vaginal term breech deliveries with a higher incidence in primigravidas compared to 0 44% in vertex vaginal deliveries Smith & Oldham (39) reported injuries in 4 of 106 cases and Rovinsky et al (35) found traumatic morbidity in 1 2% but this was still 12 times higher than in vertex vaginal delivery In the present series injuries were found in 19 of 288 (6 6%) primary vaginal deliveries Of these injuries 16 were possible related to feto-pelvic disproportion (Table II)

Even the incidence of injuries diagnosed during the neonatal period in a hospital does not of course reflect the true conditions since even minor trauma can be overlooked and perhaps sometimes be the cause of later signs of mental disturbance

### *Fetal cord complications*

The unpredictable prolapse of the fetal cord is a serious problem in the management of breech delivery The incidence (1 5%) in the present series is less than half that previously reported (11 14 17 32 42) However the seriousness of this complication is demonstrated by the fact that 4 of 5 children in the present series died or were injured The incidence of cord prolapse is especially high in footling presentation (19 29) and footling is therefore considered as a primary indication for cesarean section (22) In this context it may be mentioned that Rovinsky et al (35) explained their proven increase in asphyctic mortality in small infants as a possible result of a small breech infant leaving more space for either overt or covert cord prolapse to occur Randall et al (34) on the other hand found no greater risk of cord prolapse in small infants

### *Complicated labour*

It was not the incidence of different complications such as uterine inertia and bleeding that was considered to indicate the outcome of delivery Instead different parameters (e g fetal size pelvic capacity) were correlated to the incidence of complicated labour as defined above The incidence of complicated labour in the present study (44 of 340 cases or 13%) is in agreement with the 13% breech labours classified as complicated without further specification in Morley s review (27) It must be pointed out that complicated labour in the present study was not equated with a bad outcome of delivery but must be considered as a potential riskgroup for the infant implying feto-pelvic disproportion

### *Fetal size*

Complicated labour increased with increasing birth weight ($p<0\ 001$) and increasing circumference of the fetal head ($p<0\ 005$) in vaginal deliveries in this study Hall & Kohl (17) found increased mortality only when the infant weighed more than 4 500 grams In the present series the number of infants over 4 500 g was so small that the group exceeding 4 000 g could not be subdivided for statistical analysis Rovinsky et al (35) found significantly lower traumatic mortality and morbidity in the 2 500 to 2 999 g birth weight range and increased mortality and morbidity when the weight was 4 000 g or more

The distribution of the birth weights in the present series differed from other reports (Table VIII) This fact probably explains to some extent the relative high incidence of injuries found since complicated labour was much less frequent in the group with birth weights from 2 500 to 2 999 g (Table III)

### *Prolonged pregnancy*

Prolonged pregnancy in vertex deliveries doubled the perinatal mortality (24) In the present breech deliveries none of the children who died were associated with prolonged pregnancy (over 294 days) However the numbers are too small to permit any definite conclusions No significant increase of complicated labour occurred in prolonged pregnancy (Table V) This may indicate that mechanical factors are more important than prolonged pregnancy for the outcome in breech delivery

### *X ray pelvimetry*

There are not many reports analyzing specifically the pelvimetry data with regard to the outcome in term breech delivery Todd & Steer (42) gave an account of measurements of the pelvic inlet in 364 of their 1006 cases (35%) In 251 vaginal deliveries *they found one perinatal death (0 5%) in the group* with a sagittal diameter of 11 0 cm or more and 3 deaths in 52 cases (6%) when the diameter was below 11 0 cm The authors concluded that the figures indicate a border for safe delivery around 11 0 cm for sagittal inlet and 12 0 cm for transverse inlet diameters but they also pointed out that there were no rigid numerical criteria Dunn et al (11) reported X ray pelvimetry in 175 of 499 cases (35%) When the Mengert index for pelvic inlet was below 145 an increase in fetal death from 2 to 4% occurred and the incidence of injuries rose from 4 to 7% A corresponding result was found for the Mengert index of a midpelvis below 125 As their series is small and the size of the different groups is not given it is difficult to evaluate these findings Johnson (19) had X ray pelvimetry data available in 213 out of 500 cases (43%) In 43 cases questionable measurements and in 10 cases *more than moderately* contracted measurements existed Of these 53 patients 41 were delivered vaginally 12 deliveries were considered to be difficult including 2 fetal deaths and injuries in 8 other children Very complicated labour defined as in the present study can then be supposed to have occurred in 10 of 41 cases (24%) with small pelvimetric measurements This incidence of complicated labour is in fairly good agreement with the incidence of 3 cases of very complicated labour out of 20 borderline cases (15%) in the present study However the figures in the different studies are *not directly comparable because of probable dif*ferent criteria for the the definition of a small pelvic capacity and of the injuries included and because of the small number of cases

The 45% incidence of complicated labour and notably the 15% incidence of very complicated labour in borderline cases indicates that pelvic capacity should be greater in breech delivery than in vertex delivery

In vertex presentation X ray pelvimetry during labour gives important information about the position and degree and type of the moulding of the fetal head (5) It has also been shown that moulding of the pelvis occurs and that the area of the pelvic outlet can increase by as much as 20% in vertex labour (28) In breech delivery pelvimetry gives no information of these factors and the extent if any of the moulding of the fetal head and pelvis is unknown Anyhow it should be *emphasized that X ray pelvimetry is and in the* near future probably also ultrasonic pelvimetry will be of great importance to establish contraction of the pelvis At present methods of ultrasonic pelvimetry are being developed and *evaluated in* comparison to X ray pelvimetry (e g 20 25 33 43)

Since only primigravidas with *term breech pre*sentation have been investigated *in the present* study the risk for a contracted or a *borderline* pelvis in a multipara with a term *breech presen*tation cannot be evaluated The question *is if X* ray pelvimetry should be done also in all *multi*paras with a term breech presentation or *only in* multiparas with a previously complicated *labour* Rovinsky et al (35) recommended X ray *pelvi*metry in all term breech presentations *regardless* of parity size of infants previously *delivered* vaginally or of current estimated fetal *weight If* *in the future* ultrasonic pelvimetry of *both inlet* and outlet can be performed with high *accuracy* this problem can be solved since *ultrasound prob*ably involves no injuries

*Prognosis in breech presentation Feto Pelvic Breech Index*

In a series of 40 vaginal breech deliveries with birth weights over 3 640 g 5 of 6 cases with a contraction of pelvis (83%) and 6 of 34 cases with a normal pelvis (18%) were difficult (30) In another study cephalo pelvic disproportion was correctly diagnosed in 1 of 23 breech presentations by the use of a system of comparison of the relative difference in size of the pelvis (only sagittal diameter of the inlet) and fetal head both measured by ultrasound (20) The role of cephalopelvimetry in the diagnosis of disproportion in breech presentation has also been retrospectively studied by Perlmann (31) The author constructed 3 charts from postnatal measurements of the head and X ray pelvimetry The practical application of the charts is said to depend upon the accuracy of X ray cephalometry which the author considered to be sufficient for routine clinical use (31) In the present material retrospectively studied the risk for a complicated labour was calculated in regard to a combination of the two factors pelvic capacity and fetal size The study has shown that it is important to consider the combination of these two factors when one discusses the outcome in breech delivery Concerning the fetal size the weight of the infant seemed to be somewhat more important for the incidence of complicated labour than the size of the fetal skull In each case the fetal weight (F) and the smallest pelvic capacity (P) was retrospectively given score points and the sum of these (F+P) was called Feto Pelvic Breech Index If one estimates the fetal weight in breech presentation which is a problem per se the Feto Pelvic Breech Index can be used in the prognosis of outcome or more exact the risk for feto-pelvic disproportion to occur can be calculated The risk of a complicated labour defined as above for a given Feto Pelvic Breech Index can be seen in Fig 1

If in the present series cesarean section had been done in all cases with a Feto Pelvic Breech Index of for instance 5 or more the elective cesarean section rate would theoretically increase from 15% to 43% with a proportional decrease of the already small incidence of emergency cesarean section However the most important change would have been a decrease of the incidence of complicated labour in primary vaginal delivery from 14% to 8% The unpredictable serious prolapse of the fetal cord excluded in this example would of course decrease with increasing cesarean section rate Because of the small number of deaths the decrease in mortality cannot be statistically determined but it can be mentioned that each of the 3 cases (corrected) had a Feto Pelvid Breech Index of 5 or more Since there were no deaths or injuries in elective cesarean section very complicated labour would theoretically decrease from 4 1% to 1 2% in the total of 340 breech presentations

It should be emphasized that the Feto Pelvic Breech Index presented is based upon primigravidas The importance of parity in term breech presentation is still not clear The breech scoring index of Zatuchni & Andros (46 47) is heavily weighed against the primigravida That is breech delivery in a primigravida is considered more dangerous for the infant than in a multipara Johnson (19) found higher mortality but lower incidence of injuries in multiparas while Dunn et al (11) found contrary results and Rovinsky et al (35) found no difference between primigravidas and multiparas Therefore the Feto Pelvic Breech Index would probably be of value also in multiparas

## CONCLUSIONS

The prognostic value of the Feto Pelvic Breech Index presented depends largely on the accuracy to predict the fetal weight Ultrasonic cephalometry if available should be the first method to be applied regardless of parity in term breech presentation From the biparietal diameter the fetal weight can be estimated approximately within the limits of error of the technique and the knowledge of the range of weights corresponding to different diameters (16 23 37 41 44)

If ultrasonic cephalometry is not available an X ray film of the fetus with the mother on her side and compression of the uterus can give valuable information of the size of the fetus e g by comparing with series of films where the fetal weights are known (Fernstrom personal communication) With the latest X ray equipment including an intensifier and a 100 × 100 mm camera (fluorogram) the radiation dose for one film can be as low as 0 02 rad

Clinical palpation for prediction of the fetal weight is a third method which after systematic training possibly can be of sufficient accuracy but

it must be remembered that there is a tendency to underestimate the weight of a large infant

In all primigravidas with term breech presentation pelvimetry should be done X ray pelvimetry is presently the only accurate method for determination of the size of both the pelvic inlet and outlet Ultrasonic measuring of the true conjugate can be achieved (20) and recently the interspinous diameter has been determined (43)

In multiparas X ray pelvimetry is recommended if prediction of the fetal weight indicates a large infant or if a previous delivery has been complicated

When pelvimetry data and estimated fetal weight are known in a primigravida with a breech presentation at term it has to be decided bearing in mind all contributory factors (as e g maternal toxaemia and prolonged pregnancy) if elective cesarean section is indicated The diagram of calculated increased risk for a complicated labour (Feto Pelvic Breech Index) (Fig 1) might be a useful aid in the management of term breech presentation

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*Submitted for publication Nov 2? 1973*

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Acta Obstet Gynec Scand 54 153–156 1975

# URGE INCONTINENCE IN WOMEN

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*Abstract* A survey has been presented of the author s personal methods to treat urge incontinence in women caused by neurogenic disturbances or by urethritis De nervation of the bladder by unilateral or bilateral resec tion of the inferior hypogastric plexus is used for neuro-genic disturbances with uninhibited bladder contractions or hypertonic bladders and in cases of interstitial cystitis if a preoperative blockade with local anaesthesia has given a favourable result. Urethral diverticula are ex tirpated Urethritis is treated with careful dilatation of the urethra and massage in combination with local treat ment of the mucosa with 1% solution of silver nitrate In cases with a narrow urethral orifice a meatotomy is made Women with wide external urethral orifice and recurrent urethritis following intercourse are operated upon A structure similar to the frenulum of the pre puce in the male is constructed which closes the orifice at the introduction of the penis

In urge incontinence it is usually pain that forces the patient to evacuate the bladder in order to get relief from discomfort In cases suffering from un inhibited neurogenic bladders contractions of the detrusor muscle are often felt in a similar way Cystitis urinary calculi and ovarian carcinoma can give the same symptoms but the most common cause of urge incontinence in women is according to our experiences urethritis In our gynaecological department 2–4% of the lying in patients suffer from chronic or recurrent urethritis and among the out patients the figure is higher Urinary calculi and infections will not be dealt with in this paper with the exception of non specific urethritis

Recent anatomical studies with a fluorescent method for the detection of catecholamines in tis sues have shown that the innervation of the blad der and the urethra is much more complex than was previously realised A supply of preganglionic sympathetic nerves has been detected The blad der wall contains both sympathetic and parasym pathetic ganglia and separately adrenergic and cholinergic ganglion cells They are located pe ripherally in close relation to the innervated tis sues along the smaller nerve bundles and within the neuroterminal plexuses in the bladder musculature The majority of parasympathetic ganglion cells are encircled by a pericellular plexus of postganglionic sympathetic fibres The sympathetic ganglion cells are also probably surrounded by a corresponding network of postganglionic parasympathetic fibres As the bladder has such a complete nerve supply it is easy to understand that the function of an autonomous bladder is often superior to that of a neurogenic bladder where higher centres are still partially functioning

*Urge incontinence of neurogenic origin*

*Etiology* The etiology varies In young women multiple sclerosis may be the cause In elderly women damage to the central nervous system caused by diabetes and arteriosclerosis is common In all ages radiculitis of the sacral nerve roots as well as peripheral neuritis is found Herpes zoster in the sacral region can also give similar symptoms

*Diagnosis* A complete urological examination must be carried out A cystometry curve with con tinuous recording must be made The patient s first desire to micturate bladder capacity and sphincter pressure are recorded and the residual urine is measured Urethroscopy is very important for a correct diagnosis in order to exclude urethritis The shape and the movements of the internal urethral orifice as well as a study of the contrac tion of the middle part of the urethra can give valuable information about neurogenic disturb-ances The sensitivity of the bladder mucosa should be tested with the tip of the cystoscope

The Bartholomew Mosse Lecture 9th Nov 1973

Normally a woman can indicate on the abdominal wall where the bladder mucosa is touched In neurogenic disturbances the ability to localize is often disturbed A common observation is that touching the left side in the bladder is felt on the right side and vice versa Areas of the bladder base lacking sensitivity are sometimes found The sensitivity of the perineal region should also be tested as well as the presence of the anal reflex on touching the perineum or the labia

If uninhibited contractions are present on the cystometric curve if the bladder is hypertonic if the first desire to micturate appears before the bladder has been filled to 150 ml or if a reduced bladder capacity is found the following test should be carried out 20 ml of 0 25 per cent lidocaine solution with adrenaline 0 0005 per cent is injected into the anterior fornix 1 cm lateral to the cervix and at a depth of about 3 cm If 0 25 per cent solution is not available 0 50 per cent concentration can be used The injection is first made on one side After 5 minutes a new cystometric curve is recorded and the same tests are carried out The patient then walks around for a few minutes before she micturates and the residual urine is tested She can herself now judge about the effect of the blockade on her subjective symptoms

If a good result is obtained following the uni al anaesthesia the investigation is stopped It then evident that denervation of the inferior gastric plexus on one side will improve or cure the patient

If uninhibited contractions are still seen on the cystometric curve or if the bladder capacity is still too small 20 ml of the same lidocaine solution are injected in the same manner on the other side and the tests are carried out again

If a favourable result is obtained now and the residual urine does not exceed 150 ml there may be an indication for bilateral resection of the plexus If there is a considerable amount of residual urine only unilateral resection is allowed

*Treatment* Treatment with anticholinergic drugs should always be tried If the result of conservative treatment is unsatisfactory surgery is recommended when anaesthesia of the inferior hypogastric plexus has given a favourable result

The resection of the inferior hypogastric plexus as a method to treat these patients was discovered by chance I had a patient with severe urge incontinence in whom a Wertheim operation had to be done because of carcinoma of the cervix When I observed that her bladder functioned normally afterwards I started to study the problem systematically and a special operation was worked out

My first case was a young woman suffering from urge incontinence because of uninhibited bladder contractions caused by multiple sclerosis The pelvic anatomy proved to be completely normal The operation was therefore easy to perform and a bilateral resection was followed by complete cure

The indications for the operation were worked out empirically and preliminary results were reported 1951 and 1952 A detailed survey of 34 patients who had been operated on was presented in 1959

*Indications for surgery* Failed conservative treatment and a residual urine not exceeding 150 ml following local anaesthesia of the inferior hypogastric plexus

*Material* During the years 1950–1969 a series of 66 patients has been operated upon and followed up Two separate studies have been made namely for the years 1950–1958 in 1959 comprising 34 cases and for the years 1960–1969 recently giving a series of 32 In the first series the age varied between 15 and 62 in the latter between 39 and 85 The early series contains a group of young patients suffering from multiple sclerosis and the latter a number of old patients with bladder disturbances because of diabetic or arteriosclerotic changes in the central nervous system In the first series only 4 suffered from a combination of stress and urge incontinence In the latter 20 had this combination The differences in age distribution reflect our present aim to help the women with arteriosclerotic bladder disturbances

The first group also contains 3 cases of contracted bladders following interstitial cystitis and the latter includes 2 such cases

### *Operative technique*

The operation is a quick and easy procedure which can be combined with all kinds of surgery for urinary incontinence The technique is as follows (Ingelman Sundberg 1959)

1 A transverse incision is made below the external urethral orifice

2 The anterior vaginal wall is dissected free up to the cervix

3 A long clamp or a long pair of scissors are now pushed into the tissue between the bladder ligaments (pelvic fascia) and the levator ani and opened

4 Long retractors are introduced into the tissue at the same place and separated The nerves are then often immediately exposed appearing laterally to the rectum and following the inferior vesical vessels medially If necessary further dissection is performed by using two long dissecting forceps

5 When the nerves are laid free they are grasped between two long clamps and resected If the nerve fibres are difficult to isolate from the vessels both structures can be resected at the same time The diatermy electrode is then put on the clamps and the tissue grasped in them electrocoagulated

6 If the operation is to be bilateral the corresponding steps are carried out also on the other side

7 The vaginal wall is replaced and sutured provided no operation for stress incontinence is needed Otherwise this procedure is carried out first

8 A suprapubic catheter is introduced and the vagina is packed for 24 hrs The catheter is removed at the same time as the pack

*Results* In the first series there were 3 primary failures and in the latter only one The rate of recurrences was 15% in the first group and 6% in the latter probably reflecting improvement in technique The earliest recurrence occurred after 2 months and the latest after 5 years The only postoperative complications recorded in the series are some urinary infections

In a separate series of 8 patients 4 of them more than 70 years old and in bad general condition and 4 with recurrences after previous resection blockade with alcohol has been tried instead of operation All of them were cured primarily but recurrences occurred in all of them after 6–8 months Alcohol injection into the inferior hypogastric plexus must therefore be regarded as a palliative procedure only In unexperienced hands it may also cause damage because of the proximity to the ureter

### *Urge incontinence caused by urethritis*

During 1971–1972 38 of our gynaecological patients had such severe symptoms from non gonorrhoeic chronic or recurrent urethritis that they needed treatment in hospital 10 of them (26 3%) were 50 years old or less 21 (55 3%) between 51 and 65 and 7 (18 7%) older than 65 years Complicating diseases were stress incontinence (13) neurogenic incontinence (5) urethral diverticulum (4) urethral caruncle (3) genital prolapse (2) and diabetes (1)

*Etiology* Urethritis can be caused by many different bacteria and viruses foreign bodies and chemical substances Urethral diverticula are often the cause of a recurrent or chronic urethritis In many cases bacteria are difficult to isolate as they are concealed within the paraurethral ducts the female prostate

*Diagnosis* The history is characteristic with urgency and pain located in the urethra but also sometimes appearing as low back pain On palpation from the vagina the urethra is tender and thickened Urethroscopy reveals a red and swollen mucosa in chronic cases often with pseudopolyps floating in the stream of liquid Gonorrhoea must always be excluded

The urethral sphincter pressure is high Values of 50–60 cm of water are measured at rest and 70–80 when the patient tries to hold her urine Spasm of the sphincters gives increased resistance to micturition A bulb-like dilatation of the proximal urethra is therefore seen on X ray pictures taken during micturition

*Treatment* If a specific causative infection can be found the therapy must be directed against it Diverticula must be extirpated under specific antibiotic cover

In menopausal and postmenopausal women where the mucosa is thin treatment with oestrogens is important

For the majority of patients however local treatment is necessary The aim is to empty the paraurethral ducts and to stimulate the regeneration of the mucosa

The urethra is carefully dilated to Hegar 9 With the Hegar dilator in place massage is applied to the urethra from the vagina After that a stick with a small piece of cotton turned around its end is dipped into a 1% solution of silver nitrate and rotated as it is passed through the urethra Finally 10 ml of a colloidal silver solution is introduced through the urethra This treatment is repeated every second or third day in all 3–5 times

Finally there is a group of patients also including young women who are easily cured following the conventional therapy mentioned but get recurrences when they resume intercourse In some of these cases the husband has a urethritis which must be treated but there are patients where vaginal bacteria seem to cause the urethritis

through being forced into the urethra during coitus In a few of these the external urethral orifice is very narrow which seems to counteract the normal rinsing of the distal urethra at micturition They are usually cured following meatotomy But there are also women who have a wide external urethral meatus sometimes with caruncle formation If in these cases a mechanism could be constructed which closes the female external urethral orifice at the introduction of penis it might prevent the infection With this in mind I constructed a mechanism similar to the frenulum of the prepuce in the male and the following operation was worked out

*Operative technique* (Ingelman Sundberg 1974)

1 A transverse incision is made along the anterior vaginal circumference of the external urethral orifice If a caruncle is present it is excised

2 The anterior vaginal wall is dissected free from the urethra for a length of about 25 mm

3 From the tissue beneath the vaginal epithelium a trouser like structure is formed about 15 mm long and 2 mm thick Each leg should be 3 mm wide at the base and about 10 mm long A chromic catgut 0000 suture is placed in the tip of each leg whereupon this is trimmed laterally to a width of about 1 mm at the end The proportions must however be varied according to the local circumstances in each individual case

4 A tunnel is made under the skin of the introitus with a pair of scissors

5 The legs of the new frenulum are brought through the tunnel each one into the corresponding incision in the lateral circumference of the external urethral meatus and sutured superficially in the posterior half of the incision at its closure

6 The transverse incision under the external urethral meatus is closed

*Results* In the series of 38 cases of urethritis treated 1971–1972 for recurrent or chronic urethritis 15 were cured 21 improved and 1 not improved at the follow up one year later The frenulum operation has been made in 7 cases In 5 the results were good and 2 were failures because the frenulum did not function properly

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*Submitted for publication Dec 1 1973*

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Acta Obstet Gynec Scand 54 157–160 1975

# SEROTONIN 5 HIAA TOTAL ESTROGEN AND PREGNANEDIOL EXCRETION IN URINE DURING THERAPEUTIC SALINE ABORTION

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*Abstract* In 24 patients who underwent therapeutic abortion for various reasons between the 17th and 26th week of pregnancy urinary excretion of serotonin 5 HIAA total estrogens and pregnanediol were measured before during and after the intra amniotic injection of hypertonic saline 20% hypertonic saline solution (160–500 ml) was given by transabdominal injection over a period of 5 min The four hormones or metabolites were measured during six periods I 12–24 hrs and II 0–12 hrs before saline administration III 0–12 hrs after saline administration IV 0–12 hrs during abortion V 0–12 hrs and VI 12–24 hrs after abortion The results point to the active participation of serotonin in the process of fetal expulsion as serotonin was increased by over 100% (from 20–22 to 43–47 μg/12 hrs) during periods III–IV and its metabolite 5 HIAA too increased by nearly 60% (from 2 4–2 5 to 3 3–3 9 mg/12 hrs) They decreased during the post abortive periods V–VI On the other hand total estrogens decreased only slowly but continuously during all 6 periods (4 9 4 3 3 4 3 1 1 8 and 1 4 mg/12 hrs) Pregnanediol beginning with 12 mg/12 hrs showed a slight increase during periods III–IV (14 5 and 15 6 mg/12 hrs) and a decrease during periods V–VI (8 0 and 5 8 mg/12 hrs) These findings are interpreted as indicating the disruption of feto placental function affecting estrogens during periods III–VI They might demonstrate an accelerated hydrogenation of progesterone into pregnanediol during periods III–IV followed by a sharp decrease in progesterone/pregnanediol production during periods V–VI

Therapeutic abortion after the 14th week of pregnancy can be accomplished within 12–48 hours by replacing the amniotic fluid with sterile aqueous 20% sodium chloride solution The mechanism which provokes uterine contractions is poorly understood It may be due to mechanical stimulation (8) direct myogenic and/or spinal reflex mechanisms (6) mechanoreceptors in the uterus (19) which activate oxytocin release (1) osmoreceptors of the hypothalamus which react to the increased NaCl concentration by vasopressin release (27) dominance of placental estrogens which contract the myometrium (20) breakdown of placental progesterone which normally blocks spontaneous myometrial activity (7) release of bound prostaglandin F2 alpha which is abundant in female amniotic fluid (13) or destruction of amniotic monoamine oxidase (MAO) which metabolizes uterine serotonin to its inactive metabolite 5 HIAA (2) This plethora of possibilities induced us to study the changes in urinary serotonin 5 HIAA total estrogens and pregnanediol excretion elicited by hypertonic saline abortion

## MATERIAL AND METHODS

The histories of the 24 women studied in this trial are summarised in Table I together with the quantities of 0% normal saline given in each case Aseptic transabdominal aspiration of 0– 00 ml of amniotic fluid was carried out under local 1% lidocaine anaesthesia followed by slow replacement by 160–500 ml of sterile 0% sodium chloride solution Uterine contractions commenced in 5–7 min and resulted in abortion within 10–50 hrs

Urine was collected on 10 ml of 5N HCl i e 18–19% hydrochloric acid during six consecutive 12 hr periods

I 12–24 hours before treatment
II 0–12 hours before treatment
III 0–12 hours after treatment
IV 0–12 hours during abortion
V 0–12 hours after abortion
VI 1 –24 hours after abortion

Each patient had her urine samples examined for serotonin (16 23) and 5 HIAA (15 –1) with the 1 nitroso-2-naphthol reagent and for total estrogens (18) as well as pregnanediol (23) Values for these four parameters

Table I *Synopsis of 24 patients who underwent therapeutic abortion by transabdominal injection of 20% sodium chloride into the amniotic sac*

| No | Name | Age | Indication | Week of preg nancy | Aspira-tion fluid (ml) | NaCl 20% injected (ml) | Interval between injection of NaCl and abortion (hrs) |
|---|---|---|---|---|---|---|---|
| 1 | DH | 34 | Missed abortion VI/IV | 26 | 200 | 400 | 18 |
| 2 | EM | 28 | Coxarthrosis & X ray treatment | 22 | 150 | 400 | 32 |
| 3 | AR | 30 | Polyhydramnios & malformation | 26 | 80 | 400 | 11 |
| 4 | PH | 45 | Post X radiation | 20 | 120 | 350 | 23 |
| 5 | SG | 42 | Toxic goitre | 21 | 50 | 300 | 26 |
| 6 | IH | 43 | Severe hypertension | 21 | 100 | 500 | 10 |
| 7 | CE | 39 | Thyrotoxicosis | 18 | 100 | 350 | 28 |
| 8 | NF | 16 | Mental retardation | 17 | 80 | 400 | 37 |
| 9 | MA | 31 | Missed abortion | 25 | 50 | 300 | 23 |
| 10 | BZ | 22 | Depression | 22 | 150 | 350 | 50 |
| 11 | GZ | 23 | Post X radiation | 22 | 50 | 300 | 44 |
| 12 | BE | 22 | Rubella | 20 | 50 | 300 | 36 |
| 13 | AP | 22 | Rubella | 19 | 100 | 160 | 37 |
| 14 | PM | 33 | Rubella | 22 | 50 | 300 | 21 |
| 15 | DR | 24 | Rubella | 22 | 50 | 350 | 20 |
| 16 | RR | 28 | Rubella | 18 | 60 | 360 | 20 |
| 17 | SS | 27 | Rubella | 20 | 180 | 240 | 28 |
| 18 | RV | 28 | Rubella | 23 | 100 | 350 | 22 |
| 19 | GR | 26 | Rubella | 21 | 50 | 300 | 31 |
| 20 | KH | 31 | Rubella | 21 | 100 | 270 | 19 |
| 21 | VP | 32 | Rubella | 23 | 50 | 200 | 37 |
| 22 | CZ | 21 | Rubella | 19 | 100 | 250 | 29 |
| 23 | SE | 18 | Rubella | 19 | 100 | 350 | 48 |
| 24 | MK | 24 | Rubella | 21 | 50 | 350 | 32 |
| Average | | 39 | | 21 | 90 | 325 | 28 |

were checked during a week preceding admission to hospital and were similar to those obtained during periods I and II

## RESULTS

Table II shows the urinary excretion of serotonin 5 HIAA total estrogens and pregnanediol during the 6 periods of observation Serotonin showed a significant pattern of change after injection of NaCl (period III) it increased twofold remained at these levels throughout abortion (period IV) but receded 12 hours later (period V) and increased again 24 hours later (period VI) From that time on it tapered off slowly over a period of 1–3 days 5 HIAA behaved in a manner similar to serotonin it increased by nearly 60% after injection of NaCl

Table II *Mean urinary excretion ±S E M of serotonin 5 HIAA total estrogens and pregnanediol during 6 periods of 12 hourly collection from 24 patients during therapeutic abortion induced by 20% sodium chloride solution*

| Period no | Condition related to abortion | Interval (hrs) | Serotonin (µg per 12 hrs) | 5 HIAA (mg per 12 hrs) | Total estrogens (mg per 12 hrs) | Pregnanediol (mg per 12 hrs) |
|---|---|---|---|---|---|---|
| I | Before saline | 12–24 hrs | 20 0±1 2 | 2 5±0 3 | 4 9±0 4 | 11 6±0 6 |
| II | | 0–12 hrs | 22 0±1 3 | 2 4±0 2 | 4 3±0 3 | 1 4±0 7 |
| III | After saline | 0–12 hrs | 47 ±1 8 | 3 9±0 4 | 3 4±0 | 14.5±0 8 |
| IV | During abortion | 0–12 hrs | 43 0±1 5 * | 3 3±0 3 | 3 1±0 2 | 15 6±0 8 |
| V | After abortion | 0–12 hrs | 24 7±1 4 | 2 3±0 2 | 1 8±0 2 | 8 0±0 4 |
| VI | | 12–24 hrs | 30 ±1 6 * | 2 3±0 | 1 4±0 1 | 5 8±0 3 |

Statistical significance in difference from two control periods I and II $P<0\ 05$ $P<0\ 001$

(period III) then fell off slowly during abortion (period IV) and after abortion returned to normal values during periods V and VI Estrogens decreased immediately though not very significantly after the injection of NaCl (period III) and during all subsequent periods IV–VI Pregnanediol increased slightly after the injection of NaCl (period III) and during abortion (period IV) Later it decreased significantly to half the original values in periods V and VI

## DISCUSSION

The most spectacular result of the present study was the sharp increase in serotonin from normal values during the pre abortive periods I and II to double normal values during the periods of abortion III IV and VI We feel that this reflects the contributive role played by serotonin during abortion (17) and in normal delivery (16) Serotonin is released intra amniotically during pregnancy and constantly kept on low level by local monoamine oxidase production At the end of pregnancy the monoamine oxidase level decreases critically thus allowing an increase of uterine serotonin This serotonin surplus induces delivery (2 14 15 16 22) It is surely not the only factor initiating delivery as our own experiments to delay term in pregnant rats by serotonin antagonists have shown (21) The fact that oxytocin does not necessarily rise during normal delivery (4 5) should make us look for fetus ejectors other than oxytocin which are regularly increased during abortion (22) and during delivery (14) This touches upon the role of serotonin as well as that of the prostaglandins (9) Whether both of them are related to each other by a second or third messenger relationship remains to be investigated Gustavii & Green (12) have shown that extra amniotic injection of 20% saline solution is immediately followed by the release of prostaglandin $F_{2\alpha}$ into the amniotic fluid However they conclude that the question of whether the release of prostaglandin is the cause or the consequence of increased uterine activity must await further research Serotonin values receded during period V This may be due to its expulsion from the uterine cavity together with the conceptus (15)

5 HIAA followed the pattern set by serotonin it increased by almost 60% during the abortive periods III and IV and returned later to normal levels

The simple pattern of estrogen decrease during all six periods of saline abortion follows the accepted rules for its production and levels Quantitatively total estrogen levels are considered significant for the development of human pregnancy yet the wide fluctuations in its excretion are well known As it is synthetized jointly by the feto placental unit any significant decrease in its level is pathognomonic for threatening abortion (11) On the other hand its dominance at the end of pregnancy heralds delivery (25)

Pregnanediol poses a more complicated problem since its increase during the abortive periods III and IV may point to an accelerated metabolism of progesterone which facilitates abortion by decreasing uterine tonus Indeed the administration of intra amniotic hypertonic saline produces a fall in plasma progesterone levels (26) Progesterone causes hyperpolarization of the myometrial cell its rapid removal increases the excitability and conductivity of the myometrium and exposes it to all contractile stimuli (3) This fact does not contradict the various theories for the role played by progesterone during fetal expulsion (10)

## ACKNOWLEDGEMENTS

We wish to thank Miss D Strykowski Mrs I Juster Mrs M Zonenshein and Mrs H Peleg of the Endocrine Laboratory Rambam Hospital for devoted help in performing the estriol and pregnanediol assays

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*Submitted for publication January 4 1974*

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# IMMUNOHISTOCHEMICAL DEMONSTRATION OF CHORIONIC GONADOTROPHIN IN TROPHOBLASTIC TUMORS

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*Abstract* Chorionic gonadotrophin has immunohisto chemically been localized to trophoblastic tissue In nor mal placenta immunoreactivity was found in syncytio-trophoblastic cells However in trophoblastic neoplasms chorioadenoma destruens and choriocarcinoma the hor mone was also found in cytotrophoblastic cells The tumor cells showing immunoreactivity appeared to have distinct light microscopic features

Since the studies of Kido in 1937 (5) it is known that human placental tissue produces chorionic gonadotrophin (HCG) (1) With the aid of immuno histochemistry Midgley & Pierce (7) showed that HCG immunoreactivity was localized exclusively in the syncytiotrophoblastic layer This finding was confirmed by others (6 3 4) In addition Thiede & Choate (9) found HCG immunoreactivity in the cytotrophoblastic cells

Tumors arising from trophoblastic tissue hyda tidiform mole chorioadenoma destruens and chorio-carcinoma secrete HCG (1) Midgley & Pierce (7) localized the hormone in these tumors to syncytio-trophoblasts using the direct immunofluorescence method This method which utilizes fluorescent labelled hormone antibodies is of lower sensitivity than the indirect method (2) In the indirect method a second layer of fluorescent labelled antibodies directed against non labelled hormone antibodies is used Thereby the first layer acts as an antigen to the second layer and thus increases the total amount of labelled antibody bound to the tissue antigen (cf ref 8) It seemed of interest to study the occurrence of HCG in trophoblastic tumor tis sue using the indirect immunohistochemical meth od

## MATERIAL AND METHODS

Tumor tissue was obtained from three choriocarcinomas at hysterectomy and from two choriocarcinomas and one chorioadenoma destruens at curettage In addition normal placental tissue obtained from three induced abortions of 14– 0 weeks of gestational age was used as control tissue for immunohistochemical staining Sev eral pieces from the above mentioned tissues were fixed in 10% formalin paraffin-embedded and sectioned at 6 μ Pieces of normal placental tissue were also fixed in Bouin s fluid The sections were deparaffinized in xylene carried down to water through graded ethanol solutions refixed in Bouin s fluid for hours (see Re sults) and subjected to an indirect immunohistochemi cal method (?) for the demonstration of chorionic gonadotrophin After rinsing in phosphate buffered saline pH 7 ? (PBS) the sections were incubated for 30 min at room temperature with a rabbit antiserum directed against HCG diluted 5 times with PBS (first layer) The sections were repeatedly washed with PBS before ap-plying the second layer consisting of a fluorescein iso-thiocyanate labelled goat antiserum directed against rab-bit IgG (Miles England) diluted 0 times with PBS Af ter thorough rinsing in PBS the sections were mounted in buffered glycerine (pH 7 ?) The controls were treated as follows (*a*) the first layer was omitted (*b*) the sec ond layer was omitted (*c*) both layers were omitted (*d*) the first layer consisted of rabbit non immune serum and (*e*) the first layer consisted of the rabbit anti HCG serum containing 10 μg/ml HCG (HCG-inactivated anti serum)

The preparations were examined in a Leitz Orthoplan fluorescence microscope equipped with a Ploem system illuminator (standard filter setting position 3) The light was generated from either a halogen lamp or a HBO ?00 mercury lamp

After examination in the fluorescence microscope the sections were restained with hematoxylin-eosin







*Fig 1 (a)* Immunohistochemical demonstration of HCG in chorioadenoma destruens Note occurrence of cytoplasmic immunofluorescence (nucleus dark) in a large number of tumor cells *(b)* Hematoxylin-eosin restaining of the same section revealing a homogenous basophil cytoplasm of the immunoreactive cells ×?80

## RESULTS AND COMMENTS

In order to demonstrate HCG immunoreactivity the method of fixation proved to be important Poor results were obtained with tissue fixed in formalin partly due to high intensity of the back ground fluorescence Specimens fixed in Bouin s fluid invariably gave satisfactory results When sections from specimens previously fixed in formalin *were refixed in Bouin s fluid the results were as* good as with tissue primarily fixed in Bouin s fluid

In sections from normal placental tissue stained with the anti HCG serum strong immunofluorescence was noted in the syncytiotrophoblastic layer Tissue from chorioadenoma destruens on the other hand showed strong HCG immunoreactivity in both single cells and in syncytial formations (Fig



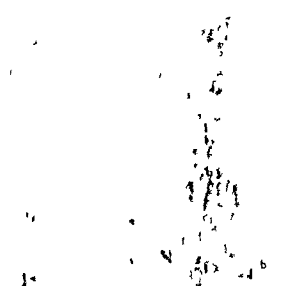




*Fig 2* Immunohistochemical demonstration of HCG in choriocarcinoma Note the occurrence of immunofluorescence in both syncytial *(a)* and unicellular elements *(b)* ×?30

1 *a*) The cells tended to be grouped together at the periphery of the villi but were sometimes seen scattered between non fluorescent cells Sections from choriocarcinomas showed immunofluorescence of moderate intensity (Fig 2) almost exclusively confined to the cytoplasm of syncytial cells often giving the impression of giant cells (Fig 2*a*) As in chorioadenoma destruens the immunofluorescent cells were usually interpositioned between non fluorescent cells In choriocarcinomas as well as in chorioadenoma destruens immunoreactive cells were often fewer than the non reactive cells In different parts of the same tumor the immunofluorescent cells varied in number and were sometimes seen to outnumber the non fluorescent cells Controls (see material and methods) were negative In one of the choriocarcinomas studied no HCG immunoreactivity could be found although a high urinary excretion of HCG was present The most likely explanation of this is rapid turnover of the hormonal product without hormone storage within the neoplastic cells However in this special case it was impossible to exclude fixation damage that may have altered the antigenicity of the hormone Midgley & Pierce (7) reported the highest intensity of immunofluorescence in choriocarcinomas In our series the opposite was a consistent finding We are unable to explain this difference

Sections of chorioadenoma destruens and choriocarcinoma *treated for the immunohistochemical* demonstration of HCG were restained with hematoxylin eosin It was found that the cells showing immunofluorescence had a homogenous and basophil cytoplasm a feature which distinguished them from the non fluorescent cells (Figs 1 *a* and *b*)

In normal placentae we found that HCG immunoreactivity was restricted to the syncytiotrophoblast In trophoblastic tumor tissue immunoreactivity was found in both syncytio and cytotrophoblastic elements This is in contrast to the findings of Midgley & Pierce (7) who observed HCG immunoreactivity only in the syncytiotrophoblastic elements of the tumors The most plausible explanation for this discrepancy is that their method has a lower sensitivity than the technique employed by us Thus cytotrophoblasts at least under pathological conditions may gain the ability to store HCG It should be noted that the immunohistochemical demonstration of a hormone producing cell depends on the hormone being present in sufficient amounts within the cell Conceivably many of the cells in these tumors that did not exhibit immunofluorescence may have the capacity of synthesizing and secreting but not storing the hormone

## ACKNOWLEDGEMENTS

This study was supported by the Ford Foundation (grant No 680-0383 A) Riksföreningen mot Cancer and J and A Persson s Foundation

The antiserum against HCG was a generous gift from Dr U Lundkvist Pharmacia Sweden The antiserum was raised in rabbits by the intramuscular injection of 3 000 I U HCG (Pregnyl Organon) in Freunds complete adjuvant Five injections were given by the same route with three weeks interval 14 days after the last injection the rabbits were bled

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*Submitted for publication Jan 11 1974*

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## REQUEST FOR DATA

The Food and Drug administration s OTC Panel is interested in receiving any unpublished data on fetal damage or loss associated with the maternal use of vaginal chemical contraceptives or douches Write to Elizabeth B Connell M D Chairman OTC Contraceptives and Other Vaginal Drug Products Review Panel FDA Bureau of Drugs Division of OTC Drug Evaluation (HFD 510) 5600 Fishers Lane Rockville Maryland 20852 USA

Acta Obstet Gynec Scand 54 165–172 1975

# EFFECTS OF TERBUTALINE ON HUMAN UTERINE MOTILITY AT TERM

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*Abstract* The effects of the selective $\beta_2$ receptor stimulator terbutaline on the activity of gravid human myometrium were investigated in vitro and in vivo before and after administration of different $\beta$ receptor blockers Terbutaline 0 2–1 0 μg/ml inhibited the spontaneous contractile activity of isolated strips of myometrium This effect was unaffected by the selective $\beta_1$ receptor blockers practolol 1 μg/ml and H 93/'6 1 μg/ml However the non selective blocker propranolol 0 1 μg/ml completely inhibited the terbutaline effects The in vitro effects of terbutaline could be correlated with findings in vivo Intra uterine pressure was recorded in 4 pregnant women at term Infusion of terbutaline 10–15 μg/min for 20–40 min effectively inhibited both spontaneous and oxytocin stimulated uterine activity There was a moderate increase in maternal heart rate but no consistent effect on maternal blood pressure Fetal heart rate was little affected The uterine effects of terbutaline were not influenced by practolol 5–20 mg i v but completely inhibited by propranolol 1–2 mg i v The results suggest that terbutaline inhibits uterine motility by effects on uterine $\beta_2$ receptors and that it can be given in clinically effective doses without adverse circulatory effects on mother or fetus

Sympathomimetic drugs are known to influence the activity of the human uterus at term (8 19) Adrenergic substances with stimulating effects mainly on α-receptors e g noradrenalin increase uterine tone and frequency and amplitude of uterine contractions (19) whereas those exerting their effects through stimulation of $\beta$ receptors e g isoprenaline have the opposite effects (17) $\beta$ receptor stimulation involves effects not only on uterus but also actions on e g heart bronchi and peripheral vessels clinically resulting in tachycardia bronchodilation and hypotension However in animals Lands et al (15) showed that the $\beta$ receptors mediating the cardiac effects ($\beta_1$) can be separated from those associated with uterine and bronchial relaxation and with peripheral vasodilatation ($\beta_2$) These findings have largely been confirmed in humans

The development of adrenergic $\beta$ receptor stimulators with effects mainly on $\beta_2$ receptors has led to an extensive use of these drugs as bronchodilators and has evoked new interest also in their clinical use as uterine relaxants Terbutaline which may be classified as a selective $\beta_2$ receptor stimulator (18) was previously shown to effectively inhibit the spontaneous contractile activity of isolated gravid human myometrium (1) The present investigation was undertaken in order to further investigate the in vitro effects of terbutaline on human uterus before and after blockade with different $\beta$ receptor blocking agents and to correlate these findings with effects in vivo Therefore the effects of terbutaline on spontaneously active and oxytocin stimulated human uterus at term was studied in patients by means of intrauterine pressure recordings (5) Also in vivo the interaction between terbutaline and the $\beta$ receptor blockers propranolol and practolol was investigated

## MATERIALS AND METHODS

*In vitro* Nineteen strips from the lower uterine segment were obtained from 7 patients aged 22– 9 years undergoing caesarean section at term The strips were dissected and mounted in organ baths as previously described in detail (1) Mechanical activity was recorded by means of force transducers (Grass Ft 03)

*In vivo* The uterine activity of 4 pregnant women at term was continuously monitored for 3–5 h A sponge tipped catheter (5) with an outer diameter of 1 8 mm was inserted through the cervical canal and placed in the uterine cavity between the membranes and the uterine wall Changes in intra-uterine pressure were recorded by means of a pressure transducer (Hewlett Packard model

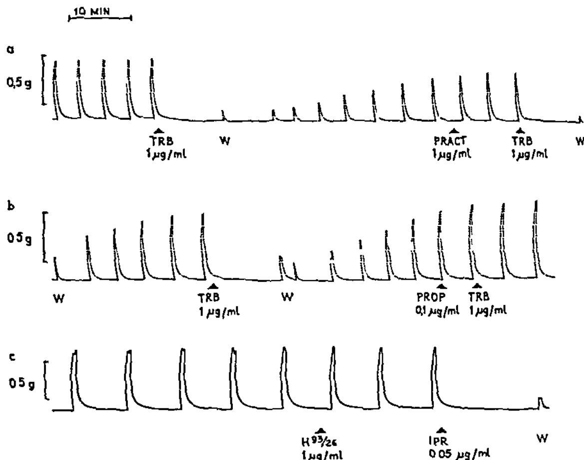



*Fig 1* Effects of terbutaline (TRB) and isoprenaline (IPR) on isolated uterine strips (*a*) Terbutaline 1 µg/ml inhibits the spontaneous contractile activity This effect is not prevented by practolol (PRACT) 1 µg/ml (*b*) Propranolol (PROP) 0 1 µg/ml completely inhibits the effects of terbutaline 1 µg/ml (*c*) H 93/ 6 1 µg/ml has no effect on the inhibitory action of isoprenaline 0 05 µg/ml W =washing

1280B) on a cardiotocograph (Hewlett Packard model 80 0A) The intensity of uterine contractions was measured as the rise in intra uterine pressure in mmHg Frequency was expressed as the number of contractions per 10 min Uterine activity was expressed in Montevideo Units and calculated for each 10 min period Fetal heart rate was recorded on the cardiotocograph using an ultrasonic flow detector (Hewlett Packard) Maternal blood pressure and heart rate were monitored throughout the investigation by percutaneous insertion of a polyethylene catheter (Stille Werner Sweden) into the radial artery The catheter was connected to a pressure transducer (EMT 746 Elema Schonander Sweden) and the blood pressure was recorded by means of an ink jet recorder (Mingograph 81 Elema Schonander Sweden)

Oxytocin and terbutaline were infused intravenously by means of an infusion pump (LKAC 501 AGA Sweden) through catheters placed in forearm veins Propranolol and practolol were injected intravenously for 2-5 min Before the administration of terbutaline propranolol and practolol uterine activity was recorded for a control period of at least 1 h

*Drugs* The following drugs were used oxytocin (Syntocinon Sandoz Switzerland) ±terbutaline sulphate (Draco Sweden) *d l* propranolol chloride (ICI England) practolol (ICI England) H 93/ 6 tartrate 1-4 methoxyethyl) phenoxy 3 isopropylaminopropan 2-ol (Hässle Sweden)

## RESULTS

### *In vitro*

After mounting in the muscle baths all strips used for the experiments developed spontaneous contractile activity during an initial period of stabilization (1–2 h) In accordance with previous findings (1) terbutaline (0 2–1 0 µg/ml) and isoprenaline (0 02–0 05 µg/ml) were found to diminish the frequency and amplitude of the spontaneous contractions in the concentrations used the drugs usually abolished the contractile activity The drug effects

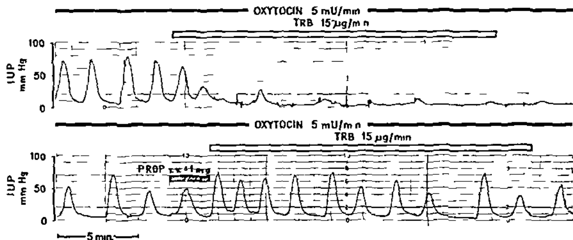



*Fig 2* Patient I Original record of intrauterine pressure (IUP) Infusion of terbutaline (TRB) 15 µg/min effectively inhibits oxytocin stimulated (5 mU/min) uterine contractions (upper panel) Terbutaline infusion has no effect after pre treatment with propranolol (PROP) 1 mg i v (lower panel)

were reversible and after repeated washing the preparation gradually increased its activity until control level was attained (Fig 1)

The effects of the selective $\beta_1$ receptor blocker practolol were investigated in 12 preparations The drug was added to the bathing fluid to a final concentration of 1–10 µg/ml and was left in contact with the preparations for 10–20 min Practolol in these concentrations had by itself no effect on the spontaneous contractile activity of the strips In a concentration of 1 µg/ml practolol had no effect on the actions of terbutaline and isoprenaline (Fig 1 *a*) However in concentrations of 5 and 10 µg/ml practolol reduced but never abolished the inhibiting effects of the β-receptor stimulators

H 93/26 a new selective β receptor blocker (21) had no effects on the spontaneous contractions of 9 myometrial strips when tested *in the concentration* range 1–10 µg/ml H 93/26 in a concentration of 1 µg/ml did not affect the actions of terbutaline and isoprenaline (Fig 1 *c*) on the strips but similar to practolol in the concentrations 5 and 10 µg/ml it reduced but never abolished the effects of these drugs

Propranolol blocking both $\beta_1$ and $\beta_2$ receptors did not affect the spontaneous activity of the preparations The effects of both terbutaline and isoprenaline were completely inhibited by propranolol *0 1* µg/ml (Fig 1 *b*) when this drug was added to the bath either before or after the administration of the β receptor stimulators The results with propranolol agree with previous findings (1)

*In vivo*

*Patient I* (31 year-old gravida I weighing 46 kg) The patient arrived at the delivery department at term with spontaneous labour and intact membranes The cervix was effaced and dilated 4 cm Stimulation of the uterine activity with oxytocin infusion 5 mU/min was started and maintained throughout the investigation Fig 2 gives the original record of intra uterine pressure changes The patient had strong uterine contractions reaching a peak of 60–70 mmHg every 2 min Infusion of terbutaline 15 µg/min had a prompt effect on the contractions Their amplitude was depressed to less than 10 mmHg and the frequency was reduced to one contraction in 4–5 min Fig 3 summarizes the different measurements of uterine contractile activity and the effects on blood pressure and heart *rate of mother and fetus* Uterine activity expressed in Montevideo Units diminished from 345 to a minimum of 15 There was a rise in maternal heart rate from 60 to about 100 beats/min but without obvious effects on maternal blood pressure The patient tolerated the infusion well and reported no side effects Fetal heart rate rose from 150 beats/min before the terbutaline infusion to about 160 beats/min and were then steady at this level

The terbutaline infusion was maintained for 20 min After cessation of the infusion the contractility of the uterus gradually returned and after 1 h and 50 min the activity expressed in Montevideo Units reached about 65 % of the pre infusion value

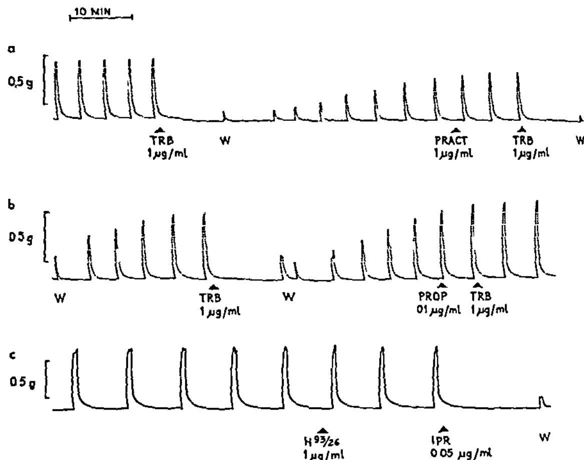



*Fig 1* Effects of terbutaline (TRB) and isoprenaline (IPR) on isolated uterine strips (*a*) Terbutaline 1 μg/ml inhibits the spontaneous contractile activity This effect is not prevented by practolol (PRACT) 1 μg/ml (*b*) Propranolol (PROP) 0 1 μg/ml completely inhibits the effects of terbutaline 1 μg/ml (*c*) H 93/ 6 1 μg/ml has no effect on the inhibitory action of isoprenaline 0 05 μg/ml W = washing

(280B) on a cardiotocograph (Hewlett Packard model 80-0A) The intensity of uterine contractions was measured as the rise in intra uterine pressure in mmHg Frequency was expressed as the number of contractions per 10 min Uterine activity was expressed in Montevideo Units and calculated for each 10 min period Fetal heart rate was recorded on the cardiotocograph using an ultrasonic flow detector (Hewlett Packard) Maternal blood pressure and heart rate were monitored throughout the investigation by percutaneous insertion of a polyethylene catheter (Stille Werner Sweden) into the radial artery The catheter was connected to a pressure transducer (EMT 746 Elema Schonander Sweden) and the blood pressure was recorded by means of an ink jet recorder (Mingograph 81 Elema Schonander Sweden)

Oxytocin and terbutaline were infused intravenously by means of an infusion pump (IVAC 501 AGA Sweden) through catheters placed in forearm veins Propranolol and practolol were injected intravenously for 2-5 min Before the administration of terbutaline propranolol and practolol uterine activity was recorded for a control period of at least 1 h

*Drugs* The following drugs were used oxytocin (Syntocinon Sandoz Switzerland) ±terbutaline sulphate (Draco Sweden) *d l* propranolol chloride (ICI England) practolol (ICI England) H 93/ 6 tartrate 1-(4 methoxyethyl) phenoxy 3 isopropylaminopropan 2-ol (Hassle Sweden)

## RESULTS

### *In vitro*

After mounting in the muscle baths all strips used for the experiments developed spontaneous contractile activity during an initial period of stabilization (1-2 h) In accordance with previous findings (1) terbutaline (0 2-1 0 μg/ml) and isoprenaline (0 02-0 05 μg/ml) were found to diminish the frequency and amplitude of the spontaneous contractions in the concentrations used the drugs usually abolished the contractile activity The drug effects

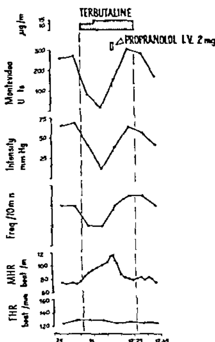



*Fig 5* Summary of the effects of terbutaline and propranolol on uterine activity maternal blood pressure and heart rate and on fetal heart rate

of the uterine activity was observed Maternal blood pressure and heart rate showed no significant alterations except for a small decrease in heart rate immediately after the injection of propranolol Fetal heart rate decreased from 160 to 140 beats/min immediately after the propranolol injection but returned to 160 beats/min after a few minutes

Delivery of a normal 3 0 kg infant occurred 5 5 hours after the end of the last infusion

*Patient 2* (22 year-old gravida II weighing 63 kg)

The patient was in spontaneous labour and the cervix was effaced and dilated 5 cm when recording of uterine activity was started The membranes were intact Fig 4 shows the original record of intrauterine pressure changes and Fig 5 summarizes the observations made Contractions occurred every 2 5 to 3 min with an average intensity of 65 mmHg Infusion of terbutaline 10 $\mu g$/min produced a decrease in the intensity of the contractions to an average of 45 mmHg and a reduction in frequency to one contraction every 4 5 to 5 min After 9 min the infusion rate was increased to 15 $\mu g$/min and this reduced the intensity of the contractions to less than 10 mmHg The frequency did not change markedly Maternal heart rate increased during the infusion from 78 beats/min to a maximum of 116 beats/min The patient tolerated the infusion well and reported no side effects except for slight palpitations After 24 min 2 mg of propranolol was injected during 2 5 min the terbutaline infusion rate being maintained at 15 $\mu g$/min This resulted in an increase in uterine contraction frequency uterine activity expressed in Montevideo Units temporarily increased to a higher level than before the terbutaline infusion Maternal heart rate was reduced after propranolol administration and reached the pre infusion value about 10 min after the injection Maternal blood pressure was measured by auscultation every 3 min during the investigation and showed no alterations Fetal heart rate was not affected by the infusion of terbutaline or the injection of propranolol

Delivery of a normal 2 4 kg infant occurred 4.5 h after the end of the terbutaline infusion

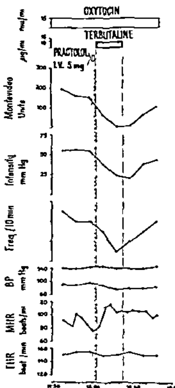



*Fig 6* Patient 3 Summary of the effects of terbutaline infusion (10 $\mu g$/min) on uterine activity maternal blood pressure and heart rate Uterine activity is stimulated with oxytocin 15 mU/min Practolol 5 mg i.v has no effect on the action of terbutaline

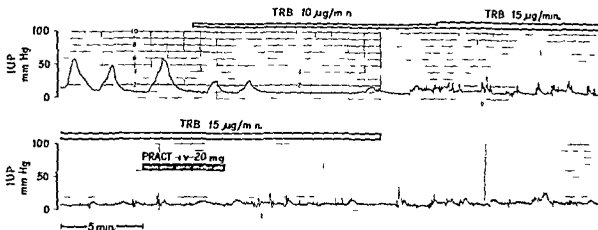



*Fig 7* Patient 4 Original record of intrauterine pressure (IUP) Infusion of terbutaline (TRB) 10–15 µg/min effectively inhibits uterine activity (upper panel) Practolol 20 mg i v has no effect on this action Cf the effects of propranolol (Fig 4)

*Patient 3 (28 year old gravida II para I weighing 69 kg)*

The patient was in labour maintained throughout the investigation with infusion of oxytocin 15 mU/min Fig 6 summarizes the different observations made The uterine contractions occurred approximately every 2 5 min and had an average intensity of 55 mmHg The cervix was at this moment dilated 4 cm Practolol 5 mg was injected intravenously during 2 min immediately followed by infusion of terbutaline 10 µg/min for 20 min The infusion produced a marked decrease in the uterine activity from 160 to less than 10 Montevideo Units (Fig 6) There was a moderate increase in maternal heart rate to a peak value of 116 beats/min but no obvious effects on the blood pressure Fetal heart rate was steady at about 150 beats/min

The patient reported no side effects during the infusion

After cessation of the terbutaline infusion the uterine work gradually increased but had not reached its pre existing activity after 30 min mostly because the contractions had a lower intensity than before the infusion Maternal heart rate did not decrease when the infusion was stopped but was unchanged for at least 30 min

After rupture of the membranes there was a normal delivery of a 3 8 kg infant 2 h after the end of the infusion

*Patient 4 (21 year-old gravida I weighing 76 kg)*

The patient was in spontaneous labour with intact membranes and the cervix dilated 4 cm when recording of uterine activity started Fig 7 shows the original recording of intrauterine pressure changes and Fig 8 summarizes the different observations made Contractions occurred every 3 min with an average intensity of 45 mmHg Infusion of terbutaline 10 µg/min was started and

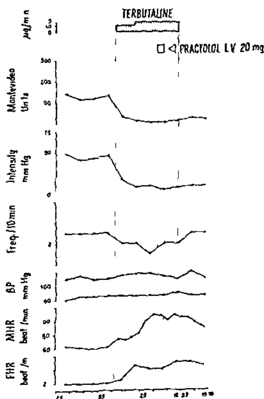



*Fig 8* Patient 4 Summary of the effects of terbutaline and practolol on uterine activity maternal blood pressure and heart rate and on fetal heart rate

the infusion rate was increased to 15 μg/min after 15 min The infusion produced a decrease in uterine activity from 134 to 10 Montevideo Units mainly due to a decrease in the intensity of the contractions There was an increase in the maternal heart rate from about 65 beats/min to a peak value of 108 beats/min Fetal heart rate increased from 120 to a maximum of 150 beats/min After about 30 min practolol 20 mg was injected at a rate of 4 mg/min during maintained terbutaline infusion There were no obvious changes in the uterine activity maternal heart rate blood pressure and fetal heart rate The patient reported no side effects during the infusion

When the terbutaline infusion was stopped after 45 min the frequency of the contractions returned to the pre existing level but they showed the same low intensity as during the infusion for a further 25 min Maternal blood pressure and fetal heart rate were unchanged but the maternal heart rate gradually decreased to 84 beats/min during the period of recording

Normal delivery of a 3 4 kg infant occurred 4 5 h after the end of the infusion

## DISCUSSION

Adrenergic β receptor stimulators have been used clinically for several years in order to produce inhibition of premature and term labour (see e g 13 16) Agents such as isoprenaline and orciprenaline which have marked effects on the cardiac β receptors ($\beta_1$) in doses necessary for effective inhibition of uterine motility also produce maternal tachycardia and hypotension and increase the risk of cardiac arrhythmias (17 20) This limits the clinical value of these drugs

Several β receptor stimulators reported to produce uterine relaxation with only minor cardiac effects have been investigated clinically e g ritodrine (4 12 14) fenoterol (Th 1165a) (9) and salbutamol (16) Terbutaline has been shown to stimulate mainly $\beta_2$ receptors (18) and is one of the most selective $\beta_2$ receptor stimulators presently available (7 11) The drug is widely used clinically in the treatment of bronchial asthma and is able to produce bronchodilation without significant cardiac effects (see e g 2 3) In a previous investigation on isolated strips of gravid human uterus (1) it was demonstrated that the β receptors of human myometrium could most probably be classified as $\beta_2$ receptors and that terbutaline effectively reduced the frequency and amplitude of the spontaneous contractile activity of this tissue The present results confirm these findings

When tested in vitro the selective $\beta_1$ receptor blockers practolol (10) and H 93/26 (21) did not influence the inhibiting effects of terbutaline and isoprenaline except when used in high concentrations On the other hand propranolol blocking both $\beta_1$ and $\beta_2$ receptors completely inhibited the effects of the two amines Also in vivo it could be demonstrated that terbutaline effectively inhibited spontaneous as well as oxytocin induced labour Both the frequency and the intensity of the uterine contractions were diminished and uterine activity expressed in Montevideo Units was reduced to less than 10 per cent of the control value Similar to the findings in isolated myometrial strips propranolol made the uterus relaxed by terbutaline resume and even increase its activity above the control level and pretreatment with propranolol completely inhibited the relaxing action of terbutaline In contrast practolol did not affect the actions of terbutaline on uterine motility Thus there was a good correlation between the effects of terbutaline and the β receptor blockers on isolated uterine tissue and on the uterus in situ

During infusion of terbutaline there was a tolerable increase in maternal heart rate but no significant effects on maternal blood pressure The positive chronotropic effect which can be expected to be a main side effect when high doses of terbutaline are given can probably be ascribed not only to a direct stimulation of the cardiac β-receptors but also to a reflex adjustment to peripheral vasodilatation (2) Though practolol had no definite effect on the increase in heart rate produced by terbutaline in the 2 patients tested it may be possible to control excessive tachycardia with $\beta_1$ receptor blockers such as practolol and H 93/26 without interfering with the uterus relaxing effect of terbutaline In support of this view tachycardia produced by ritodrine was successfully controlled by practolol (6)

In 3 of our 4 cases no consistent effect on fetal heart rate was observed during infusion of terbutaline but in one there was a moderate increase from 120 to 150 beats/min

Although preliminary the present results suggest that effective inhibition of uterine activity at term can be obtained with terbutaline without ad-

verse effects on maternal or fetal circulation Further clinical evaluation of the effects of terbutaline in premature and term labour is in progress

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*Submitted for publication January 29 1974*

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Acta Obstet Gynec Scand 54 173–181 1975

# THE EFFECT OF A COPPER IUD AND INERT IUDS ON THE INCORPORATION OF $^3$H THYMIDINE AND 5 $^3$H URIDINE INTO THE ENDOMETRIUM OF THE RABBIT AFTER STIMULATION WITH HUMAN CHORIONIC GONADOTROPIN

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*Abstract* The present investigation studied the influence of different types of intra uterine devices (IUDs) especially that of a copper *IUD* on the incorporation of $^3$*H* thymidine and 5 H-uridine into the endometrium of the rabbit In ert polyethylene IUDs had no effect on the incorporation of these labelled precursors The effect of the copper IUD was always compared with that of an in ert IUD In non stimulated rabbits the incorpora tion of $^3$H thymidine was increased in the copper in fluenced horn The incorporation of $^3$H thymidine in control and Cu IUD-containing horns reached a maxi mum at 48 hours after HCG stimulation but was sig nificantly lower in the copper-containing horn than in the control horn On the fifth day of pseudopregnancy the incorporation of $^3$H thymidine was significantly higher in the copper IUD-containing horn The total amount of DNA in the endometrium increased during early pseudopregnancy *but this increase was markedly reduced* in the presence of copper The copper IUD had no influ ence on the rate of incorporation of 5 $^3$H uridine in non stimulated rabbits whereas it caused a higher incorpora tion on the fifth day of pseudopregnancy

Possible modes of action of IUDs have been re viewed recently (6 8 9 10 11 15 19 25 26 27 28) The nucleic acid metabolism of the uterus is one of the parameters studied In women an inert IUD (Margolis spiral or Lippes loop) increased the amount of RNA in the endometrium (17) A slight increase in the RNA content was also observed in wearers of the Cu T (12) No alteration in the amount of DNA in the endometrium was found in this in vestigation However the DNA content per cell nucleus was decreased in the proliferative phase in cells obtained with brush technique (13) Reports on the nucleic acid content in the uterus of other species are somewhat contradictory An inert IUD had no influence on the RNA or DNA content in the uterus of the Rhesus monkey (18) During early pregnancy in the rat an intra uterine silk suture had no effect on the content of RNA and DNA (20) whereas a nylon IUD significantly increased the amount of these constituents (29) The DNA content in the uterus was lowered in the presence of a silk suture in ovariectomized estrogen and progesterone treated rats (16) The Indian water buffalo showed an increase in the amount of *RNA* in the uterus in the presence of a polyethylene IUD (14)

Estrogen treatment of ovariectomized rats fitted with intrauterine silk sutures resulted in a higher incorporation of the radioactivity of $^{14}$C glucose into the RNA in the IUD wearing horn (21) whereas the *incorporation of $^{32}$P was uninfluenced (16)* When ovariectomized rats were treated with estrogen and progesterone the incorporation of the radioactivity of $^{14}$C-glucose into RNA decreased (21) whereas the incorporation of $^{32}$P into RNA of the IUD horn remained at the same level as in estrogen treated animals and decreased in the control horn (16) A copper IUD significantly decreased the incorpora tion of $^{14}$C thymidine into DNA of the endometrium in normal rats stimulated with a large dose of estrogen (24) A decreased incorporation was also observed in the presence of a zinc IUD but the copper IUD was more efficient A nylon thread had no significant effect in this experimental system

IUDs can obviously induce changes in the metabolism of nucleic acids in the uterus in many species under different hormonal conditions How ever some of the results are contradictory for ex ample the discrepancy in the DNA content in the endometrium and in the endometrial cell

ported by Hagenfeldt (12 13) and the studies on the incorporation of labelled precursors described by Joshi (16) and Laumas & Yadava (21) Furthermore the effects of inert and copper IUDs have only rarely been studied in the same experimental system

The aim of the present investigation was to develop in the rabbit an experimental model suitable for studying the effect of various inert IUDs and a copper IUD on the metabolism of nucleic acids in the endometrium

## MATERIAL AND METHODS

Seventy six virgin rabbits of mixed breed weighing between two and three kg were used in this study Four types of IUDs were studied

1 Copper—consisting of a 50 mm long polyethylene catheter 0 5 mm in outer diameter (Clay Adams Parsippany N Y USA) around which a copper wire 0 23 mm in diameter was coiled giving a surface area of 200 mm² (*Cu IUD*)

2 Platinum—identical with the Cu IUD except for the change of metals (*Pt IUD*)

3 Plastic—consisting of the same polyethylene catheter 0 5 mm in outer diameter and 50 mm long as described above (*Pl IUD*$_{0.5}$)

4 Plastic—consisting of a polyethylene catheter 1 0 mm in diameter and 50 mm long (*Pl IUD*$_{1.0}$)

The IUDs were sterilized by soaking in ethanol and rinsed in sterile physiological saline before use The animals were anaesthetized with Nembutal and the abdominal cavity was exposed under sterile conditions via a lower midline incision No corpora lutea (C L ) were seen at the time of operation The IUDs were introduced into the uterine horns via a small puncture in the antimesometric part of the uterine wall just above the utero-cervical junction and the lower end of the IUDs were fixed to the uterine wall by means of a 4-0 silk suture at the site of the puncture Uterine horns designated as sham operated (SH) were exposed to introduction and withdrawal of the Pl IUD$_{1.0}$ and a 4-0 silk suture was placed in the antimesometric wall The effect of the different IUDs was compared with the effect of the Pl IUD$_{1.0}$ Thus one horn was always fitted with a Pl IUD$_{1.0}$ and the other with one of the other types of IUDs in all groups except the group where one of the horns was sham-operated The IUDs were always randomized between the horns Tables I-III list the experimental groups with the number of animals in each subgroup On the fifth day after operation the animals were given 75 IU of HCG (HCG human chorionic gonadotropin Gonadex Leo AB Helsingborg Sweden) intravenously to induce pseudopregnancy The animals were then killed by cervical dislocation 48 72 and 120 hours after the injection Some animals—killed on the fifth day after operation corresponding to 0 hours—were not injected with HCG

The internal genitalia were taken out and the uterine horns were trimmed The presence of C L in each ovary was recorded The uterine horns were opened by an incision along the mesometrial wall and the endometrial surface was gently rinsed with Parker 199 The endometrium of the uterine horns *corresponding to the length* of the IUD (called the IUD part) was thoroughly scraped with a sharp curette and immediately put into 2 ml Parker 199 SBL Stockholm Sweden) at 37°C The tissue was then brought into a suspension of small tissue fragments and single cells with the aid of a stainless steel mesh through which the tissue was *gently pressed with a pestle (according* to Borell 1952) The endometrial cell suspension was then divided into two to four equal portions and centrifuged for five minutes at 300 g The supernatants were discarded and the pellets resuspended in 0 5 ml prewarmed Parker medium containing either ³H thymidine (³H TdR thymidine methyl-³H Schwarz/Mann Inc Orangeburg NY specific activity 1 9 C/mM) at a final concentration of 10 μC/ml or Uridine 5 ³H (5 ³H UR NEN Frankfurt a m West Germany specific activity 27 8 C/mM) at a final concentration of 20 μC/ml The cells were incubated at 37°C for one hour under continuous agitation with a magnetic stirrer The tubes were then centrifuged at 300 g for 5 minutes The pellets were washed in phosphate buffered physiological saline pH 7 1 extracted in five per cent TCA at 4 C for 30 minutes and washed once in TCA and once in absolute ethanol The samples were then stored at −20°C until further processed The incubations were set up within 90 minutes after the killing of the animals All tests were carried out in duplicate

### *Determination of DNA and radioactivity*

The cells were dissolved in 0 6 or 1 0 ml 1 N sodium hydroxide for one hour at 37°C This solution was used for determination of DNA according to the method of Cenotti (1952) as modified by Bonting & Jones (1957) A standard curve of five double samples was made for each series of determinations with calf thymus DNA (B D H Chemical Division Poole England) The angle coefficient of the standard curve was calculated and the amount of DNA in the test solution expressed as the extinction value ($OD_{490}$) was divided by the angle coefficient This value is called the adjusted DNA value

To determine the amount of radioactivity 100 μl of the sodium hydroxide solution was transferred to a scintillation vial To this was added 1 ml of Soluene 100 (Packard Ltd Stockholm Sweden) and 14 ml of scintillation solution (300 mg dimethyl POPOP 5 g PPO 1000 ml toluene) The determinations were then carried out in a Packard TriCarb spectrometer 3310 The efficiency was almost constant and was continuously checked with external standardization

### *Statistical considerations*

As demonstrated by Nordqvist (1969) the cpm/DNA values are not normally distributed but a near normal distribution can be obtained by logarithmation Thus as a measure of the incorporation of the different labelled precursors the following expression was used

$$a = 100 \times \log_e \frac{\text{cpm} \times 10^4}{\text{AES} \times (\text{DNA})}$$

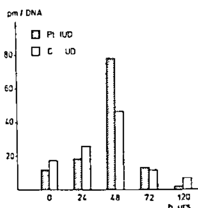



*Fig 1* The diagram shows the mean values of the incorporation of ³H TdR into DNA in endometrial cells of Pl IUD , and Cu IUD-containing horns at various times after HCG stimulation The rate of incorporation is expressed as cpm/DNA

where (DNA) is the adjusted DNA value in OD ₂₆₀ units and AES the value of the automatic external standard The statistical calculations were performed on these *a* values In the diagrams the mean values of the incorporation of labelled nucleosides are expressed as cpm/DNA (*a* values divided by 100 and anti logarithmated) which permits a direct estimate of the differences

The effects of the various IUDs were evaluated as follows One horn was always fitted with the Pl IUD the other horn with another type of IUD or was sham operated The *a* value for each horn (the mean of double values) was determined and the difference between these values for the two horns of each animal was calculated If two different IUDs influence the incorporation of the labelled precursors in the same way and to the same extent the *a* values will be about the same for the two horns and the differences between them will scatter around zero The deviation from zero was tested by an analysis of variance where all subgroups (different times after stimulation of the animals with HCG) in each experimental group were included The analysis of variance was performed according to the following formula

$$F=\frac{(Q_s+n x\ )/n}{Q_d/(N\ n)}$$

where $Q_s$ is the sum of squares between subgroups $x$ is the mean value of all differences in the group $Q_d$ is the sum of squares within subgroups N is the total number of animals in the group and $n$ is the number of subgroups

If the differences of a group were found to deviate significantly from zero the deviation of the mean differences of each subgroup from zero can be analysed by means of a two sided $t$ test (Armitage 1971)

$$t=\frac{x}{\sqrt{Q_d/(N\ n)}\ \ \sqrt{1/n}}$$

where x is the mean value of the differences of the subgroup $n$ is the number of animals of the subgroup $Q_d$, N and $n$ are as above

These statistical methods were also used to analyse differences in the total amount of DNA in horns fitted with different IUDs The amount of DNA is expressed in

Table I *Effect of IUDs on the incorporation of H TdR into the DNA in endometrial cells*

The differences in incorporation between differently treated horns in each group were tested in an analysis of variance The variance ratios (*F* values) and the significance levels are shown in the table The deviation of the mean differences in each subgroup from zero was analysed by means of a *t* test the table gives the significance levels Negative mean differences indicate that the incorporation is lower in the Pl IUD -containing horns

| Group | Combination of IUDs | *F* value | *P* | Subgroups (hours) | No of animals | Mean difference | S E | *P* |
|---|---|---|---|---|---|---|---|---|
| I | Pl IUD Cu IUD | 9 33 at 5 and 15 d f | <0 001 | 0 | 3 | −15 57 | 11 10 | N S |
| | | | | 4 | 3 | −15 69 | 11 10 | N S |
| | | | | 48 | 5 | 7 74 | 8 60 | 0 01<*p*<0 0? |
| | | | | 7 | 4 | 5 6 | 9 61 | N S |
| | | | | 1 0 | 5 | −51 17 | 8 60 | <0 001 |
| II | Pl IUD , Cu IUD (Non stimulated rabbits) | 1 87 at 3 and 6 d f | 0 001<*p* <0 005 | 0 | 3 | 1 0 | 8 30 | N S |
| | | | | 48 | 3 | −49 5 | 8 30 | <0 001 |
| | | | | 170 | 3 | −14 39 | 8 30 | N S |
| III | Pl IUD SH | 10 at 3 and 17 d f | N S | 0 | 5 | 31 66 | – | – |
| | | | | 48 | 5 | −19 05 | – | – |
| | | | | 1 0 | 5 | −1 48 | – | – |
| IV | Pl IUD Pl IUD ₓ | 1 79 at 3 and 1 d f | N S | 0 | 5 | 7 36 | – | – |
| | | | | 48 | 4 | − 61 | – | – |
| | | | | 1 0 | 6 | 7 4 | – | – |
| V | Pl IUD ,-Pt IUD | 7 98 at 3 and 1 d f <0 005 | N S 0 001<*p* | 0 | 5 | 79 94 | 8 77 | 0 005<*p*<0 01 |
| | | | | 48 | 5 | 0 25 | 8 77 | N S |
| | | | | 1 0 | 5 | 70 37 | 8 7 | 0 075<*p*<0 05 |

ported by Hagenfeldt (12 13) and the studies on the incorporation of labelled precursors described by Joshi (16) and Laumas & Yadava (21) Furthermore the effects of inert and copper IUDs have only rarely been studied in the same experimental system

The aim of the present investigation was to develop in the rabbit an experimental model suitable for studying the effect of various inert IUDs and a copper IUD on the metabolism of nucleic acids in the endometrium

## MATERIAL AND METHODS

Seventy six virgin rabbits of mixed breed weighing between two and three kg were used in this study Four types of IUDs were studied

1 Copper—consisting of a 50 mm long polyethylene catheter 0 5 mm in outer diameter (Clay Adams Parsippany N Y USA) around which a copper wire 0 23 mm in diameter was coiled giving a surface area of 200 mm² (*Cu IUD*)

2 Platinum—identical with the Cu IUD except for the change of metals (*Pt IUD*)

3 Plastic—consisting of the same polyethylene catheter 0 5 mm in outer diameter and 50 mm long as described above (*Pl IUD*$_{0.5}$)

4 Plastic—consisting of a polyethylene catheter 1 0 mm in diameter and 50 mm long (*Pl IUD*$_{1.0}$)

The IUDs were sterilized by soaking in ethanol and rised in sterile physiological saline before use The animals were anaesthetized with Nembutal and the abdominal cavity was exposed under sterile conditions via a lower minal midline incision No corpora lutea (C L ) were present at the time of operation The IUDs were introduced into the uterine horns via a small puncture in the antimesometric part of the uterine wall just above the utero-cervical junction and the lower end of the IUDs were fixed to the uterine wall by means of a 4-0 silk suture at the site of the puncture Uterine horns designated as sham operated (SH) were exposed to introduction and withdrawal of the Pl IUD$_{1.0}$ and a 4-0 silk suture was placed in the antimesometric wall The effect of the different IUDs was compared with the effect of the Pl IUD$_{1.0}$ Thus one horn was always fitted with a Pl IUD$_{1}$ and the other with one of the other types of IUDs in all groups except the group where one of the horns was sham operated The IUDs were always randomized between the horns Tables I–III list the experimental groups with the number of animals in each subgroup On the fifth day after operation the animals were given 75 IU of HCG (HCG human chorionic gonadotropin Gonadex Leo AB Helsingborg Sweden) intravenously to induce pseudopregnancy The animals were then killed by cervical dislocation 48 72 and 120 hours after the injection Some animals—killed on the fifth day after operation corresponding to 0 hours—were not injected with HCG

The internal genitalia were taken out and the uterine horns were trimmed The presence of C L in each ovary was recorded The uterine horns were opened by an incision along the mesometrial wall and the endometrial surface was gently rinsed with Parker 199 The endometrium of the uterine horns corresponding to the length of the IUD (called the IUD part) was thoroughly scraped with a sharp curette and immediately put into 2 ml Parker 199 SBL Stockholm Sweden) at 37°C The tissue was then brought into a suspension of small tissue fragments and single cells with the aid of a stainless steel mesh through which the tissue was gently pressed with a pestle (according to Borell 1952) The endometrial cell suspension was then divided into two to four equal portions and centrifuged for five minutes at 300 g The supernatants were discarded and the pellets resuspended in 0 5 ml prewarmed Parker medium containing either ³H thymidine (³H TdR thymidine methyl ³H Schwarz/Mann Inc Orangeburg NY specific activity 1 9 C/mM) at a final concentration of 10 μC/ml or Uridine 5 ³H (5 ³H UR NEN Frankfurt a m West Germany specific activity 27 8 C/mM) at a final concentration of 20 μC/ml The cells were incubated at 37°C for one hour under continuous agitation with a magnetic stirrer The tubes were then centrifuged at 300 g for 5 minutes The pellets were washed in phosphate buffered physiological saline pH 7 1 extracted in five per cent TCA at 4 C for 30 minutes and washed once in TCA and once in absolute ethanol The samples were then stored at −20°C until further processed The incubations were set up within 90 minutes after the killing of the animals All tests were carried out in duplicate

### *Determination of DNA and radioactivity*

The cells were dissolved in 0 6 or 1 0 ml 1 N sodium hydroxide for one hour at 37 C This solution was used for determination of DNA according to the method of Cenotti (1957) as modified by Bonting & Jones (1957) A standard curve of five double samples was made for each series of determinations with calf thymus DNA (B D H Chemical Division Poole England) The angle coefficient of the standard curve was calculated and the amount of DNA in the test solution expressed as the extinction value (OD$_{600}$) was divided by the angle coefficient This value is called the adjusted DNA value

To determine the amount of radioactivity 100 μl of the sodium hydroxide solution was transferred to a scintillation vial To this was added 1 ml of Soluene 100 (Packard Ltd Stockholm Sweden) and 14 ml of scintillation solution (100 mg dimethyl POPOP 5 g PPO 1000 ml toluene) The determinations were then carried out in a Packard TriCarb spectrometer 3310 The efficiency was almost constant and was continuously checked with external standardization

### *Statistical considerations*

As demonstrated by Nordqvist (1969) the cpm/DNA values are not normally distributed but a near normal distribution can be obtained by logarithmation Thus as a measure of the incorporation of the different labelled precursors the following expression was used

$$a = 100 \times \log_e \frac{\text{cpm} \times 10^4}{\text{AES} \times (\text{DNA})}$$

Table III *Effect of IUDs on the total amount of DNA in endometrial cells*

Negative mean differences indicate that the total amount of DNA is lower in the Pl IUD ₒ-containing horns For further explanations see Table I

| Group | Combination of IUDs | *F* value | *P* | Subgroups (hours) | No of animals | Mean difference | S E. | *P* |
|---|---|---|---|---|---|---|---|---|
| I | Pl IUD-₁ₒ Cu IUD | 7 67 at 5 and 15 d f | 0 005<*p* <0 01 | 0 | 3 | 0 0452 | 0 1960 | N S |
| | | | | 24 | 3 | 0 2375 | 0 1960 | N S |
| | | | | 48 | 5 | 0 6705 | 0 1414 | <0 001 |
| | | | | 72 | 4 | 0 3061 | 0 1697 | N S |
| | | | | 120 | 5 | 0 4877 | 0 1414 | 0 005<*p*<0 01 |
| II | Pl IUD ₒ-Cu IUD (Non stimulated rabbits) | 1 12 at 3 and 7 d f | N S | 0 | 3 | −0 2037 | – | – |
| | | | | 48 | 3 | 0 2256 | – | – |
| | | | | 120 | 4 | −0 0232 | – | – |
| III | Pl IUD ₒ SHAM | 0 08 at 3 and 12 d f | N S | 0 | 5 | 0 0041 | – | – |
| | | | | 48 | 5 | 0 0314 | – | – |
| | | | | 120 | 5 | 0 0669 | – | – |
| IV | Pl IUD ₒ Pl $IUD_{0.5}$ | 1 68 at 3 and 14 d f | N S | 0 | 5 | −0 1470 | – | – |
| | | | | 48 | 6 | −0 0429 | – | – |
| | | | | 1 0 | 6 | 0 1014 | – | – |
| V | Pl IUD ₒ-Pt IUD | 0 80 at 3 and 12 d f | N S | 0 | 5 | −0 0397 | – | – |
| | | | | 48 | 5 | 0 0153 | – | – |
| | | | | 120 | 5 | −0 1527 | – | – |

The influence of the Cu IUD on the incorporation of ³H TdR into the endometrium of non stimulated rabbits at 0 48 and 120 hours was studied and again compared with the influence of the Pl IUD ₀ The results are shown in Fig 2 A and Table I (Group II) The rate of incorporation seems to be higher in the Cu IUD horn at 48 and 120 hours whereas no difference was found at 0 hours An analysis of variance performed on the differences including all subgroups gives a significant *F* value (0 001<*p*<0 005) The *t* tests showed significant differences only at 48 hours (*p*<0 001)

The effect of an inert IUD on the incorporation

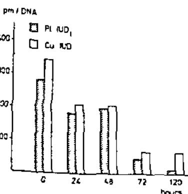



*Fig 3* The diagram shows the mean values of the incorporation of 5-³H UR into RNA in endometrial cells of Pl IUD and Cu IUD-containing horns at various times after HCG stimulation

of ³H TdR was studied in a series where the incorporation into the endometrium of Pl IUD₁ₒ containing horns was compared with the incorporation into sham operated horns (Fig 2 B) No statistically significant difference between these treatments was found (Table I Group III) However at 0 and 48 hours there were fairly large differences which might be of biological importance

The Pl IUD₁ₒ is a little stiffer and has a smoother surface than the Cu IUD To make sure that the differences in effect between Pl IUD₁ₒ and Cu IUD were not due to differences in physical properties the Pl IUD₁ₒ was compared with the effect of the Pl $IUD_{0.5}$ (which is somewhat more pliable than the Cu IUD) and the Pt IUD (which has the same surface structure as the Cu IUD) As Table I Group IV Fig 2C shows no statistically significant difference was found between the Pl IUD₁ₒ and the Pl $IUD_{0.5}$ Therefore no t tests for the subgroups were performed However there is a tendency towards a higher incorporation into the endometrium of the Pl IUD₁ₒ-containing horn at 0 hours It cannot be excluded that this difference might be of biological significance The Pt IUD caused a lower incorporation at 0 and 120 hours compared with the Pl IUD₁ (Table I Group V Fig 2 D) A comparison between the effect of the Cu IUD and the Pt IUD would reasonably have revealed even larger differences than those shown (Table I Group I)

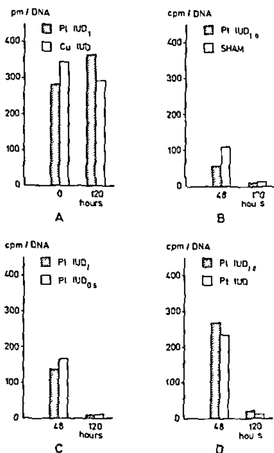



*Fig 4* The diagrams show the mean values of the incorporation of 5 $^3$H UR into RNA in endometrial cells of horns containing various types of IUDs (A) Non stimulated rabbits (B–D) Rabbits stimulated with HCG

### *ffect of IUDs on the incorporation of 5 $^3$H UR*

The incorporation of 5 $^3$H UR into the endometrium of horns fitted with either Pl IUD$_{1\ 0}$ or Cu IUD is shown in Fig 3 No stimulation of the incorporation was observed during early pseudopregnancy Instead the incorporation gradually decreased In all subgroups in this series the incorporation is higher into the endometrium of the Cu IUD-containing horns (Fig 3) An analysis of variance including all subgroups shows that the difference is statistically significant ($0\ 001<p<0\ 005$ see Table II Group I) In the $t$ tests only the difference at 120 hours was found to be significant ($p<0\ 001$ see Table II Group I) No significant differences were found in any of the other groups (Table II Groups II–V) i e the effect of sham operation Pl IUD$_{0\ 5}$ Pt IUD did not differ from the effect of the Pl IUD$_{1\ 0}$ in stimulated animals and the effect of the Cu IUD did not differ from the effect of the Pl IUD$_{1\ 0}$ in non stimulated animals (Fig 4 A–D)

### *Effect of IUDs on the DNA content in the endometrium*

The total amount of DNA in the endometrium of the IUD part of each uterine horn was determined As shown in Figs 5 and 6 the DNA content in the endometrium increased markedly when the animals were stimulated with HCG Fig 5 shows the DNA content of Pl IUD$_{1\ 0}$ and Cu IUD containing horns At 0 and 24 hours no difference was observed At 48 72 and 120 hours there was a gradually increasing difference in DNA content with more DNA in the Pl IUD$_{1\ 0}$ horn An analysis of variance on all differences in all subgroups gives a significant $F$ value ($0\ 001<p<0\ 01$ Table III Group I) When $t$ tests were performed significant differences were found in two of the subgroups at 48 hours ($p<0\ 001$) and at 120 hours ($0\ 001<p<0\ 01$)

No significant differences were found between copper IUD containing horns and horns fitted with Pl IUD$_{1\ 0}$ in non stimulated animals (Table III Group II) Nor were there any significant differences between Pl IUD$_{1\ 0}$ Pl IUD$_{0\ 5}$ Pt IUD and sham operation in stimulated rabbits (Table III Groups III–V)

## DISCUSSION

The effect of copper on the incorporation of $^3$H TdR and 5 $^3$H UR into the endometrium of the rabbit during early pseudopregnancy has been studied A copper IUD was introduced into one of the uterine

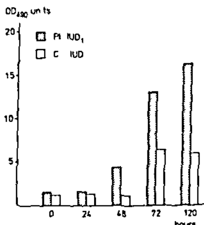



*Fig 5* The diagram shows the total amount of DNA expressed in OD$_{490}$ units for the Pl IUD and Cu IUD-containing horns at various times after HCG stimulation

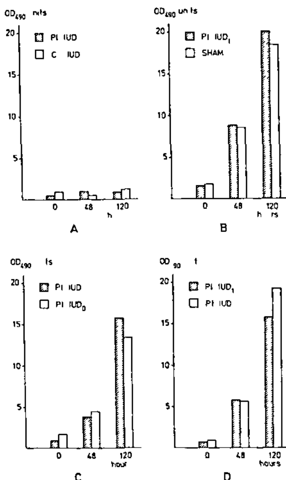



*Fig 6* The diagrams show the total amount of DNA expressed in $OD_{490}$ units for horns containing various types of IUDs (A) Non stimulated rabbits (B–D) Rabbits stimu lated with HCG

horns and as the mere presence of an inert IUD might influence these metabolic processes the other horn was fitted with a control IUD This ought to be identical with the copper IUD except for the pres ence of copper As control IUD a polyethylene tubing (the Pl $IUD_{1\,0}$) was used This is somewhat stiffer than the Cu IUD and has a different surface structure However no significant differences in the incorporation of labelled precursors were found when the influence of this IUD was compared with that of the Pl $IUD_{0\,5}$ (which is more pliable) Furthermore no significant difference was found between the incorporation of labelled precursors into Pl $IUD_{1\,0}$-containing horns and sham operated horns Some groups show fairly large non significant differences which might have some biological importance for example at 0 hours when the effect of the Pl $IUD_{1\,0}$ was compared with that of the Pl $IUD_{0\,5}$ and shamoperation The Pt IUD caused a significantly lower incorporation of $^3H$ TdR (at 0 and 120 hours) than did the Pl $IUD_{1\,0}$ The reason for this difference is obscure but had the Cu IUD been compared with the Pt IUD instead of the Pl $IUD_{1\,0}$ the registered differences would have been even larger

In non stimulated rabbits the incorporation of $^3H$ TdR was higher in the horn containing the Cu IUD No significant effect was found at 0 and 120 hours whereas the difference at 48 hours was statistically significant At present it is not known why this effect of the Cu IUD appears only on the seventh day (corresponding to 48 hours after HCG stimulation) after insertion of the IUDs As far as we know an increased incorporation of labelled nucleosides into copper influenced endometrial cells has not previously been reported The incorpo ration of $^3H$ TdR into the endometrium reached a peak at about 48 hours after HCG stimulation This peak was significantly lower in the Cu IUD contain ing horn At 120 hours the incorporation of $^3H$ TdR had decreased markedly in the Pl $IUD_{1\,0}$ horn and in the Cu IUD horn although it was significantly higher in the latter These effects on the DNA syn thesis resulted in a lesser increase in the total DNA content in the Cu IUD horn As the amount of DNA in the endometrium was determined only in the IUD part of the horns the results might be influ enced by differences in the degree of contraction in differently treated horns However this is unlikely as no differences in the DNA content of the en dometrium were found in non stimulated animals fitted with Cu IUD and Pl $IUD_{1\,0}$ or in HCG stimulated rabbits where the effect of the Pl $IUD_{1\,0}$ was compared with that of the Pl $IUD_{0\,5}$ Pt IUD or shamoperation Furthermore the amount of DNA in the Cu IUD influenced horn in HCG stimulated animals is only about 30 per cent of that in the Pl $IUD_{1\,0}$ influenced horn This large difference can hardly be due to inappropriate division of the uterine horns

Our results to some extent agree with those re ported by Prager (24) and Hagenfeldt (13) Prager found a lower uptake of $^{14}C$ thymidine into the DNA in the endometrium of estrogen stimulated normal rats in the presence of copper The labelled pre cursor was administered into the uterine lumen As the inflammatory response in the presence of copper

is more pronounced than it is in the presence of inert IUDs (7 22) the lower uptake could have been due to an increased breakdown of the precursor by polymorphonuclear leucocytes (5) or to an in creased resorption rate from the endometrium

Hagenfeldt (12) reported that the DNA content in endometrial biopsies was not influenced by the pres ence of the Cu T in women However the DNA content per cell nucleus was decreased in cells obtained by brush technique (13) These findings are somewhat contradictory but the latter agrees with our results as we found a decreased DNA content in the copper influenced endometrium

Inert IUDs did not influence the DNA content in the present study whereas both increased and decreased DNA concentrations have been reported in studies in other species under various hormonal conditions (17 18 20 29) The RNA synthesis was also influenced by the presence of the Cu IUD as the incorporation of 5 $^3$H UR was significantly higher in the Cu IUD influenced horns at 120 hours after HCG stimulation This finding agrees with the increase in RNA concentration in wearers of the Cu T (12) No significant differences in RNA syn thesis were found in the present study when inert IUDs were studied Laumas & Yadava (21) and Joshi (16) studied the effect of silk sutures in ovariectomized rats under different hormonal stimu lation and obtained contradictory results concerning the incorporation of labelled precursors into RNA The RNA concentration has been reported to be unaltered (18 20) or increased (17 29) in the pres ence of inert IUDs in different species

Exactly how IUDs exert their anti fertility effect is still a matter of controversy It is suggested that implantation is disturbed either because of a blastotoxic effect or because of changes in the en dometrium which makes it unsuitable for implanta tion (8 27 28) It is obvious from the present discus sion that IUDs can alter the nucleic acid metabolism of the endometrium in several species In the present study inert IUDs in rabbits had no such effect in contrast to a copper IUD How copper exerts this effect on the nucleic acid metabolism is not known

## ACKNOWLEDGEMENTS

We are most grateful to Dr Holger Rootzen Statistical Adviser of The Medical Research Council University of Lund Sweden for advice on the statistical treatment of the data

The cost of this investigation was defrayed by a grant from the Ford Foundation (No 640-0186 B)

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*Submitted for publication April 24 1974*

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# ANNOUNCEMENTS

*The Vth International Seminar on Reproductive Physiology and Sexual Endocrinology Sperm Action* will take place 14–17 May 1975 in Brussels Belgium Organizing Committee Professors O O Hubinont P Soupart R Schoysman J Schwers and M D M L Hermite Provisional program Wednesday May 14 Spermatogenesis and sperm transfer Thursday May 15 Testicular function Friday May 16 Fertilization Saturday May 17 Pharmacological control of male fertility Secretary Mrs P Jonart Laboratoire de Gynécologie Hôpital Universitaire St Pierre Rue Haute 322 1000 Brussels Belgium

*The VII Academic Session of German Speaking University Teachers of Obstetrics and Gynaecology* will take place in Munich West Germany on June 18–21 1975

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*Foundation of a Laqueur Medal for the VII Academic Session of German Speaking University Teachers*

On the occasion of the VII Academic Session ORGANON Munich institutes the Laqueur Medal which will be awarded to a university teacher resident in Europe for prominent and systematic clinical scientific investigations in the field of physiology and pathology of human reproduction The value is 15 000 DM

The President of the Academic Session invites German speaking university teachers of obstetrics and gynaecology resident in Europe to make nominations for the award The jury is composed of the committee preparing the session and a physician from the medical department of ORGANON Munich The jury is entitled to seek professional advice from other experts

*A Symposium with International Participation on Endocrine Regulation of Human Reproduction* to be held in *Bratislava from September 3rd to 5th 1975 is organized by* the Czechoslovak Society for Gynaecology and Obstetrics Topics 1) Mechanism of hormonal influence of cell metabolism in the reproductive organs of woman 2) LH RF FSH RF in the experiment and clinic of ovarial cycle disorders 3) Evaluation of endocrinological examinations in normal and risk pregnancies

Papers can be presented in English and German Information Slovak Medical Society Congress Office Mickiewiczova 18 883 22 Bratislava Czechoslovakia

*The IVth European Sterility Congress* will take place in Madrid October 5–8th 1975 Preliminary Program and further information can be had from the Secretariat Dr J Cortés-Prieto Puerto de Bermeo 11 Madrid (34) Spain

Acta Obstet Gynec Scand 54 183–189 1975

## RECENT OBSERVATIONS

# PERFORATION OF THE UTERUS BY THE COPPER T AND COPPER 7 INTRAUTERINE CONTRACEPTIVE DEVICES

Lars L Cederqvist Bengt Åke Lindhe and Fritz Fuchs

*From the Department of Obstetrics and Gynecology (Head Frit Fuchs) The New York Hospital – Cornell Medical Center New York and the Department of Obstetrics and Gynecology (Head Bengt Åke Lindhe) The Hospital Bollnas Sweden*

*Abstract* Uterine perforation in patients wearing the Copper T and the Copper 7 intrauterine contraceptive devices has been studied In Bollnas Sweden three perforations occurred in 1 156 insertions of the Copper 7 and in New York USA six perforations occurred in 1 153 insertions of the Copper T Cervical perforation seems to be a special feature of the Copper T while the Copper 7 tends to perforate through the uterine wall The perforations can be divided into primary perforations related to the insertion procedure and secondary perforations caused by uterine contractions The diagnosis and treatment of uterine perforations by intrauterine devices is discussed

The Grafenberg and Ota rings and their modifications representing the first generation of intrauterine contraceptive devices (IUD s) did not signal a breakthrough in contraceptive technology The second generation of IUD s on the other hand represented by the Lippes loop the Hall Stone ring the Margulies spiral and many others achieved a marked success within a short period of time Almost all of them were made of inert materials such as polyethylene or stainless steel A definite further improvement appeared to be achieved with the introduction of copper bearing devices which may be considered representational of a third generation of intrauterine devices The copper feature however seems to have fascinated the inventors so much that they forgot to pay attention to the other features of the device Both the Copper T and the Copper 7 have sharp points which can penetrate and perforate the uterine wall The Chulalongkorn Family Planning Clinic in Bangkok Thailand has observed five (four published) perforations in 1 220 insertions of the Copper T (Rienprayura et al 1973) and from The New York Hospital – Cornell Medical Center two perforations in 880 insertions have previously been reported (3) Recently six perforations of the Copper T were reported in a material of 3 000 insertions in the USA (16) One of these translocated IUD s also caused an intestinal perforation The purpose of the present paper is to report on six uterine perforations in a total of 1 153 insertions of the Copper T (CuT 200) at The New York Hospital – Cornell Medical Center New York and three perforations by the Copper 7 in 1 156 insertions at the Bollnas Hospital in Sweden

## CASE REPORTS

*Case 1* D C a 21 year-old gravida 1 para 0 underwent an elective abortion in 1971 at six weeks gestation A CuT 200 IUD was inserted at The New York Hospital Family Planning Clinic in November 197? on the ninth day of the menstrual cycle into a uterus of normal size in mid-position without any difficulty The patient had no complaints at her first check up and the tail of the IUD was protruding through the cervix On the next visit eleven months after the insertion the tail of the IUD was again found in the cervical os In addition the copper bearing tip had perforated the cervix from the upper part of the cervical canal and the tip could be seen at 7 o clock in the posterior fornix A hysterogram confirmed the cervical perforation (Fig 1) The IUD was removed through the opening in the posterior fornix and the newly formed fistula was cauterized with silver nitrate The patient was given oral contraception at her own request At later follow-up the patient was without complaint and no fistula could be identified

*Case 2* E N a 19 year-old gravida 0 was first seen at

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device was removed through the cervical os without difficulty and the patient was put on oral contraceptives

*Case 6* S Z a 29 year-old gravida 0 had a CuT 200 inserted at The New York Hospital Family Planning Clinic in March 1974 during the third day of her menstrual cycle into a normal uterus without difficulty On her first control six weeks later she had no complaints but the lower portion of the device was protruding through the cervix into the posterior fornix at 6 o clock The device was pulled through the fistula with ring forceps and the patient left the clinic in good condition She is scheduled to return for reinsertion of another device

*Case 7* I J a 34-year-old gravida 6 para 6 who had a Copper 7 inserted in September 1973 at Bollnäs Hospital on the 14th day of her menstrual cycle without any difficulty At her first check up two months after the insertion the pelvic examination was normal and the thread was protruding through the external os Four months after the insertion she returned after having missed a menstrual period Early pregnancy was confirmed and the patient underwent a

*Fig 2* Flat plate of the abdomen with a sound in the uterus indicates the Copper T to be located outside the uterine cavity This was also confirmed by hysterogram

*Fig 3* Hysterogram showing a retroverted uterus with one of the arms of the Copper T (indicated by arrow) penetrating the cervical canal

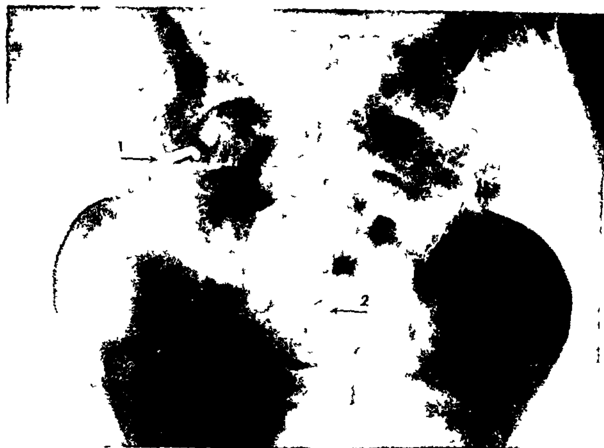



*Fig 4* Flat plate of the abdomen demonstrating two Copper 7 IUD s One is located in the right upper quadrant of the abdomen (arrow No 1) while the other (arrow No 2) is in place in the uterus

erapeutic abortion two weeks thereafter At the time of the curettage the intrauterine device could not be found A flat plate X ray of the abdomen revealed the Copper 7 to be dislocated to the right upper quadrant of the abdomen At the patient s request a new Copper 7 was inserted and two weeks thereafter the patient finally granted permission for the dislocated device to be removed by laparoscopy Fig 4 illustrates a hysterogram with the translocated Copper 7 in the right upper quadrant of the abdomen and another one in the uterine cavity

*Case 8* S S a 19 year old gravida 1 para 0 who underwent a therapeutic abortion at the Bollnas Hospital in 1973 and four weeks thereafter had a Copper 7 inserted on the fifth day of the menstrual cycle One month later she returned because of severe pelvic pain A hysterogram showed a partial perforation of the device through the uterine wall (Fig 5) The device was removed without incident and the patient was put on birth control pills

*Case 9* S St a 31 year old gravida ? para ? with her youngest child delivered in November 197? A Copper 7 was inserted one year later at the hospital in Bollnas without incident on the fourth day of the menstrual cycle Three months after the insertion the patient returned with a chief complaint of meno-metrorrhagia Hysterography demonstrated a partial perforation of the device through the uterine wall (Fig 6) The device was easily removed and the patient s menstrual pattern returned to normal

## DISCUSSION

Perforation of the uterus is one of the most serious complications of the intrauterine contraceptive devices This complication was first brought into focus by Nakamoto & Buchman in 1966 and the potential dangers were underscored by the report by Scott (12) Nakamoto & Buchman discovered five perforations of the Birnberg bow with the aid of the Beolocator an electronic detector for location of foreign bodies (4) and later found as many as 16 perforations in a total of 544 insertions of this device (1) The bow was thought to be particularly perforation prone due to its design and its spring loaded introducer Scott s inquiry among Fellows of the American College of Obstetricians and Gynecologists showed that perforations occurred with other devices as well and that perforation into the abdominal cavity of closed or




*Fig 5* Hysterogram showing a partial perforation of a Copper 7 through the uterine wall The translocated part of the IUD is marked with an arrow

ring shaped devices could lead to potentially fatal intestinal obstruction

Perforations can occur as a result of faulty insertion by which the device is pushed into or through the uterine wall such perforations may be called primary perforations They can also be due to displacement of the device through the forces of uterine contractions i e secondary perforations Most of the perforations of the Copper T are secondary caused by uterine contractions forcing the stem of the device into the cervical tissue when the angle between the corpus and the cervix is too sharp Rienprayura et al (11) observed five such downward perforations (four published) in 1220 insertions the stem of the T had perforated to the vaginal fornices in four and to the cul-de sac and recto-vaginal septum in one case A similar type of perforation had been observed by Lehfeldt & Wan (8) and in our first 880 insertions we initially observed two such perforations (3) both of which are included in the present report while Williamson & Kirkland (16) found six perforations in 3 000 insertions of the Copper T Landesman et al (7) have shown that not only the stem but also the pointed ends of the horizontal bar of the T end to penetrate into the uterine muscle In fact the low expulsion rate of the Copper T may be ascribed to the ability of the bar to impinge on the uterine walls

No perforations have so far been reported with the Copper 7 although this device shares several features with the Copper T It has two sharp points rather than three and the stem though fairly rigid due to the copper is curved and therefore better able to follow the flexions of the uterus Accordingly downward perforations are less likely to occur than with the T and this seems to be borne out by our experience described above

The true incidence of uterine perforations with intrauterine devices is not known but there is obviously a relationship between the design and the risk of perforation Peel & Potts (10) estimated the incidence of perforations per 1 000 insertions to be



*Fig 6* Hysterogram demonstrating a partial perforation of Copper 7 through the uterine wall The translocated part the IUD is marked with an arrow

approximately 0 4 for the Lippes loop 0 3 for the Margulies spiral 1 0 for the Hall Stone ring and 5–8 for the Birnberg bow Johannisson (6) has postulated that the better the conformity between the device and the uterine cavity the less the risk of perforation This is to some extent borne out by our experience with the Antigon F (4) in more than 3000 insertions we have observed no perforations at all In fact there is only a single perforation on record with the various modifications of the Antigon (5) Similarly only one perforation has been observed so far in about 5000 insertions of the Ypsilon designed by Soichet (14) On the other hand Snowden & Williams (13) have observed a number of perforations with the Dalkon Shield (approximately 1 350 insertions)

Our experience with the two copper bearing devices as reported here indicates a fairly high incidence of perforation namely six perforations in 1153 insertions of the Copper T (1 19?) and three perforations in 1156 insertions of the Copper 7 (1 385) This is much higher than the figure of 1 5000 given for the Copper T by Tatum (1973) The designers of the copper bearing devices seem to have been too pleased with the contraceptive effect of copper to pay enough attention to the other features of the design It would seem better to apply copper to devices which even without copper have a high contraceptive effect and which have shapes with the least risk of perforation In developing countries copper bearing devices are not ideal unless they retain a high degree of protection after the copper has disappeared because of the difficulties of adequate follow up

The diagnosis of uterine perforations with intrauterine devices is often difficult Disappearance of the tail from the cervical os and inability to feel the device with a uterine sound should always raise the suspicion Foreign body detectors like the Beolocator (no longer in production) and sonography may be of considerable help but so far radiography particularly hysterography has been the most useful diagnostic procedure

If an inert device perforates into the peritoneal cavity the risk of damage is small unless the device is closed or ring shaped in which case a part of the intestines may be caught and intestinal obstruction may occur Copper bearing devices irritate the peritoneum and cause adhesions They are often found imbedded in a ball of omentum Because of their sharp points intestinal perforation may occur Copper bearing devices should therefore be removed as soon as possible Even inert devices are usually removed Laparoscopy and culdoscopy have been helpful for this purpose but laparotomy or colpotomy may be required

The downward perforations which seem to be a special feature of the Copper T are less serious The device can usually be extracted through the formed fistula or it can be pushed up into the uterus and removed through the cervical canal It has been our practice to cauterize the fistula with silver nitrate and no permanent fistula has been observed so far


## ACKNOWLEDGEMENTS

This work was supported in part by Grants Nos 67-03? and 73-049 from the Rockefeller Foundation

The Copper T's were supplied by The Population Council and the Copper 7 s by G D Searle & Co Inc (Chicago)


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*Submitted for publication Aug 13 1974*


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# BOOKS RECEIVED

*Fertility and Sterility* edited by T Hasegawa M Hayashi F J G Ebling and I W Henderson International Congress Series No 278 About 900 pages Price Dfl 180 00 (about US $63 20) Excerpta Medica Amsterdam 1973

This book contains Proceedings of the VII World Congress of Fertility and Sterility in Tokyo and Kyoto October 17–25 1971 It covers the whole field of research dealing with reproduction and gives a good review concerning treatment of infertility as well as the new trends in fertility control Organ transplantation in reproduction and *in vitro* culture of the mammalian egg and its transplantation into recipients are described Professors T Hasegawa and M Hayashi have edited the part concerning human fertility and S J Ebling and J W Henderson the section about animal reproduction The book is highly recommended to the libraries of all university departments of obstetrics and gynaecology as well as to research workers in this field of reproduction

*Precis de Gynécologie* by H-G Robert R Palmer C Boury Heyler and J Cohen Masson et Cie Paris 1974 837 pp 507 ill Price about Fr 300

A modern very complete and well illustrated textbook which can be used for undergraduates as well as for post graduate students

*I S*

Acta Obstet Gynec Scand 54 191–192 1975

# LETTER TO THE EDITOR

## CORRESPONDENCE ON THE STUDY ISOLATION AND IDENTIFICATION OF CORYNEBACTERIUM VAGINALE (HAEMOPHILUS VAGINALIS) IN WOMEN WITH INFECTIONS OF THE LOWER GENITAL TRACT

In their reply (4) to my Letter to the Editor (2) Doctors Åkerlund and Mårdh write that my criticism which concerned their appraisal of the value of wet mounts Gram and Papanicolaou stained smears in diagnosing Corynebacterium vaginale (Haemophilus vaginalis) (3) was based on a complete misinterpretation of their data

What then are the data in their study on which Doctors Åkerlund and Mårdh must have based their appraisal of the value of the different types of specimens in diagnosing Corynebacterium vaginale (Haemophilus vaginalis)?

The authors do not present the data in a Table or Graph In Results subheading Findings in wet mounts Gram and Papanicolaou stained smears page 87 (3) the authors write Clue cells were most easily recognized in the Gram stained smears but were also found in the wet mounts and in the Papanicolaou stained smears In the groups of patients with LGTI the frequency of smears containing clue cells did not vary with the presence or absence of Corynebacterium vaginale in the cultures Clue cells were not found in the smears of any of the healthy controls

On inspection the results are seen to be imprecise and inadequate to be organized in tabulated form and to allow of a comparison of the findings on different types of specimens with the cultural identification of Corynebacterium vaginale (Hameophilus vaginalis) in the respective groups of women Consequently an evaluation of wet mounts Gram and Papanicolaou stained smears in diagnosing the organism is not possible

Discussing the above findings the authors reveal one new piece of information not given in the results They write on the last page of their study (3) Neither were any clue cells found in 8 out of 22 patients from whom Corynebacterium vaginale had been recovered Even if this information is added to the results the data thus combined would still be imprecise and insufficient for a detailed evaluation of the different types of direct specimens

In their letter (4) Doctors Åkerlund and Mårdh deny that Gram stained cervical secretion was used in their study in search for clue cells In this connection they refer to their study as follows As clearly described in our study (4) page 86 wet mounts were made from material from the posterior vaginal fornix A quotation of page 86 Material and Methods subheading Wet mounts Gram and Papanicolaou stained smears (3) will be found to read Discharge from the posterior vaginal fornix was mixed with a drop of saline and immediately examined as a wet mount under a light microscope Material collected from the cervical canal the portio and the posterior vaginal fornix was fixed in ethanol and stained according to Papanicolaou Cervial secretion was Gram stained The wet mounts and the smears were examined particularly for clue cells i e epithelial cells covered with numerous coccoid bacteria (3 9 15) When this information and that of the Results ( Clue cells were most easily recognized in the Gram stained smears ) are considered together it appears as the only possibility that the Gram stained smears were those prepared from the cervical secretion

Finally the authors choice to use a specimen from the cervical canal only in the identification of Corynebacterium vaginale (Haemophilus vaginalis) is likely to distort the results in that for instance in cases where clue cells are found in a vaginal specimen a negative culture of Corynebacterium vaginale (Haemophilus vaginalis) from an intracervical specimen would not exclude the possibility of the organism being present in the vagina Corynebacterium vaginale (Haemophilus vaginalis) is being associated with a vaginal but not with an intracervical condition (1)

Thus a study of the paper of Doctors Åkerlund

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Acta Obstet Gynec Scand 54 193–194 1975

## BRIEF REPORT

# IN VITRO EXPERIMENTS ON ADHERENCE OF BACTERIA TO VAGINAL EPITHELIAL CELLS

P A Mårdh L Westrom and M Åkerlund

It has been stated (1 4 6) that the occurrence of vaginal epithelial cells covered by numerous coc coid rods i e clue cells in wet mounts Papanicolaou or Gram stained smears constitute a criterion for an infection with Corynebacterium vaginale In a recent study (7 8) in which we used the isolation methods of C vaginale recommended by Dunkelberg et al (2) we were not able to con firm the diagnostic value of clue cells Thus clue cells were found in one third of a series of women with signs of infection in the lower genital tract and from whom C vaginale could not be reco vered

Bacteria may adhere to mucosal epithelial cells e g of the mouth (3) and the intestinal tract (5) Such an adherence has also been demonstrated in in vitro experiments in which oral epithelial cells have been incubated with bacteria (3)

We have studied the adherence of various rod shaped bacteria to vaginal epithelial cells in an in vitro system Vaginal epithelial cells were collected from women with no clinical signs of infection or cytological evidence of inflammation The speci mens were obtained by scraping the vaginal epi thelium with an Ayre s spatula The samples were transferred to test tubes containing phosphate buf fered saline (PBS) pH 5 5 They were then washed in fresh PBS five times through a mem brane filter with a pore size of 14 $m\mu$ (Millipore) This pore size retains cells but allows bacteria to pass

Bacteria were scraped off agar plates which had been incubated at 37°C for 18 hours and suspended in PBS To suspensions of washed cells bacteria of the following species were added Bacteroides fragilis coliform rods (Enterobacter) C vaginale Escherichia coli Fusobacterium glutinosum and Haemophilus parainfluenzae The number of cells and bacteria was determined in a Burker s counting chamber Mixtures of $10^5$ cells and $10^9$ bacteria per ml of PBS were incubated at 37°C for 30 minutes The suspensions in their syringes to which the membrane holders were adapted were placed on a shaking machine The mixtures were then washed again five times in fresh PBS through the membrane filter which allowed the bacteria not adhered to cells to pass The epithelial cells on the filter were then transferred to glass slides which were dried heated gently and methylen blue or Gram stained In additional experiments PBS of pH 4 5 or 7 2 was used throughout the procedure

Only a few lactobacilli at the most adhered to washed vaginal epithelial cells not mixed with bacteria An adherence of bacteria to the cells was demonstrated with all the species studied (Fig 1) However the experiments also indicated that the capacity to adhere varied between the species It was thus possible to produce clue cells in this in vitro system with bacteria of several species Varia tion of the pH (4 5–7 2) of the buffer did not obviously influence the adherence

Little is still known about factors that influence the contact between bacteria and vaginal cells in healthy women and in patients with genital infec tions The ability of bacteria to adhere to epithelial cells seems to be a prerequisite for the colonization of mucosal surfaces This capacity may be influ enced by environmental factors It may vary from one bacterial species to another which may to a certain extent account for differencies in virulence In the present in vitro study it was found that the bacteria studied including facultatively and strictly anaerobic rod shaped bacterial species were able to adhere to the surface of vaginal epithelial cells

and Mårdh (3) reveals that it cannot serve as a reference on the diagnostic value of wet mounts Gram and Papanicolaou stained smears concerning Corynebacterium vaginale (Haemophilus vaginalis) mainly for these reasons (1) the lack of results allowing an evaluation of the different types of specimens in diagnosing the organism and (2) the use of cervical secretion as a matherial for Gram stained smears which nullifies the results on this group of specimens

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## REPLY TO LETTFR

It is most remarkable that Dr Leppaluoto has admitted himself to basing his statements on misleading quotations in which he leaves out some clarifying points of information that we made in our first reply to him (Acta Obstet Gynecol Scand *53* 285 1974) For instance he does not quote Naturally for clue cells we looked at the smears from the portio and the posterior vaginal fornix

Dr Leppaluoto seems to be a firm believer in C vaginale being unique among rod shaped bacteria to adhere to vaginal epithelial cells Discussing the value of clue cells for the establishment of an infection with C vaginale we want to refer to our le entitled In vitro experiments on adherence of bacteria to vaginal epithelial cells published in this issue Finally we still do not believe that one can identify with certainty as to species a gram labile rod by cytological means Hereby the debate with Dr Leppaluoto is closed on our part

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Acta Obstet Gynec Scand 54 195–202 1975

# PROGESTERONE THERAPY IN PRE ECLAMPTIC TOXAEMIA

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*From the Departments of Obstetrics (Head Professor A H Makhlouf) and Pediatrics (Head Professor A Wshahi) Faculty of Medicine Ain Shams University Cairo Egypt*

*Abstract* Progesterone was the principal therapeutic agent used in 40 cases of pre-eclamptic toxaemia It was also given to 10 normal pregnant women who served as a control group A significant fall in both the systolic and diastolic blood pressures was observed in 80% of the toxaemic patients In all cases there was a marked increase in the 24-hour urinary output with an apparent loss of weight Serum uric acid showed a significant drop and the urea clearance values also improved The serum sodium showed an apparent decline while the serum potassium did not show any marked variation The serum uric acid urea clearance blood urea serum sodium and serum potassium remained unchanged in the control group

Pre eclampsia is a specific disease initiated and maintained during pregnancy and manifested by hypertension oedema proteinuria and occasionally convulsions and coma Residual effects after delivery are not necessarily sequels of this syndrome

Harrison (8) found that in cases of renal hypertension the blood pressure declined spontaneously during the last few days of pregnancy and rose again gradually after delivery until it reached the original level Corbit (2) showed that pregnancy tended to produce about a 20% reduction of the systolic pressure both in normal and in renal hypertensive rabbits The blood pressure returned to the pre pregnancy level gradually after labour Foa et al (4) found that the systolic blood pressure of renal hypertensive rats was markedly reduced during pregnancy After delivery the systolic blood pressure rose again to the pre pregnancy levels The authors suggested that the drop in blood pressure might be due to an endocrine mechanism Grollman (7) demonstrated a tendency for the blood pressure to decline during pregnancy in renal hypertensive dogs rabbits and rats but pseudo-pregnancy was not accompanied by a change in the blood pressure

Sammour in 1972 demonstrated changes in the vaginal smear of pregnancies complicated with pre eclampsia suggestive of progesterone deficiency and these changes were reversible after progesterone administration (16)

The purpose of the present investigation is to study the effect of progesterone administration on pregnancy complicated by pre eclamptic toxaemia

## MATERIAL AND METHODS

A group of 40 pregnant women with pregnancies of 34 to 40 weeks gestation and suffering from pre eclamptic toxaemia were given natural progesterone as the only line of treatment for their toxaemia This group included

1 Nineteen cases of mild pre-eclamptic toxaemia whose blood pressure varied between 135–160 mmHg systolic and 90–100 mmHg diastolic The oedema was limited to the lower limbs and their proteinuria was mild

2 Sixteen cases of severe pre-eclampsia as indicated by a systolic blood pressure above 160 mmHg and a diastolic blood pressure of 100 mmHg or more pitting oedema of the lower limbs and abdominal wall and gross albuminuria

3 Five cases of fulminating pre-eclampsia with short duration of symptoms and high blood pressure reaching over 180 mmHg systolic and 110 mmHg diastolic extensive oedema and gross albuminuria

Ten normal pregnant women at 34–38 weeks gestation were taken as control cases for studying the effect of natural progesterone administered in doses similar to those used in toxaemic patients

The progesterone was given by deep intramuscular injection in the gluteal region In order to obtain a constant high level of progesterone the drug was given in 4 divided doses of 50 mg each at six hour intervals The administration of the drug continued according to the individual clinical response the maximum duration

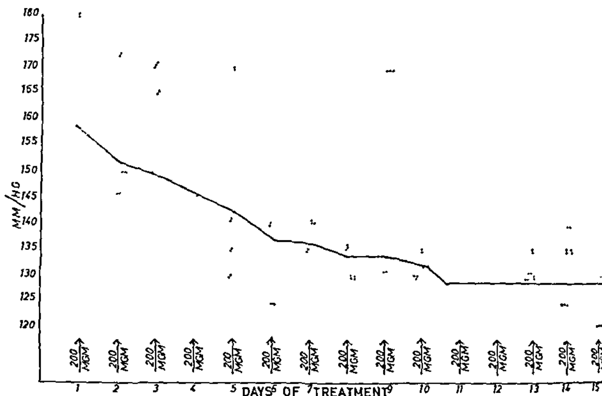



*Fig 1* Scattergram of the systolic blood pressure changes on natural progesterone therapy for toxaemic patients

being six weeks The earliest response was obtained after 36 hours from the start of treatment

Women in or beyond the 38th week of pregnancy were given the drug until the onset of labour Those at the 36th week were given the drug for two weeks and the pregnancy was then terminated by artificial rupture of the membranes

All patients were admitted to hospital and kept in bed for 24 hours during which regular recording of the blood pressure was carried out in order to register the basal blood pressure After 24 hours progesterone therapy was started In cases of fulminating pre eclampsia progesterone was administered immediately on admission

The following tests and investigations were carried out

1 *Weight* Recorded every 24 hours at 7 30 a m before breakfast The extent of the oedema was tested *daily*

2 *Blood pressure* Recorded every 6 hours in all cases

3 *Urine* The 24-hour urine output was measured and tested for specific gravity sugar pus cells and total protein content

4 *Blood urea* Estimated by the urease Nesselenza tion method before starting the treatment when the patient responded to treatment and ont the 10th day of therapy

5 *Urine urea* The urine was diluted 100 times and its urea estimated by the same method as for blood urea.

6 *Urea clearance* The urea clearance was calculated as a percentage of the normal The urea clearance test was estimated before starting the treatment when the patient responded to treatment and on the 10th day of therapy

7 *Serum uric acid* McFarlane (13) considered the level of serum uric acid to present the most sensitive test of renal function particularly with toxaemias of pregnancy Their serum uric acid level was estimated at the start of treatment and when the patient responded and on 10th day of therapy

8 *Serum sodium and potassium* These were estimated by flame phtometry before the treatment was started when the patient responded on the 7th day of therapy

## RESULTS

### *Effect of Natural Progesterone Therapy on Pre eclamptic Toxaemia*

A *Clinical response*

32 (80%) of the 40 patients with pre-eclampsia who were treated with natural progesterone showed definite clinical response

1 *Blood pressure* The earliest drop in the

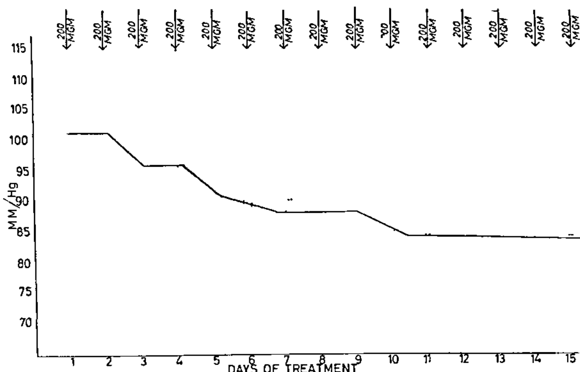



*Fig 2* Scattergram of the diastolic blood pressure changes on natural progesterone therapy for toxaemic patients

systolic blood pressure was noticed 24 hours after the onset of treatment while the maximum drop was reached on the 4th day of treatment In the 3? patients who responded to therapy there was a drop in the systolic blood pressure from a mean of 170 mmHg to 130 mmHg

Three of the five patients with fulminating pre eclamptic toxaemia responded to treatment while in the remaining two cases the response was mini *mal with no change in the blood pressure*

The drop in the systolic blood pressure was rapid during the first 3 or 4 days of treatments after which the pressure was maintained at 130 mmHg

Fig 1 shows a scattergram of the systolic blood pressure of the 32 pre-eclamptic patients who responded to progesterone therapy

There was also a drop in the diastolic blood pressure in 34 of the 40 patients who received progesterone therapy i e 85 % The earliest change appeared 72 hours after the start of treatment somewhat later than the drop of the systolic pres sure The level was then maintained at about 85 mmHg The lowest diastolic pressure was obtained on the 7th day of treatment i e the maxi mum response was achieved 3 days later than that of the systolic blood pressure

Fig 2 shows a scattergram of the changes in the diastolic blood pressure

2 *Volume of urine* All 40 patients with pre eclamptic toxaemia had a diuresis following progesterone therapy The volume of urine during the *first 24 hours under treatment was at least double* the amount passed during the preceding day with an average of 1 500 cc Diuresis was maintained throughout the course of treatment The manifest oedema of the lower limbs disappeared within a period of 7 days These patients were not con fined to bed

Fig 3 demonstrates the changes in the volume of urine as compared with the changes in blood pressure and body weight under the effect of natural progesterone

3 *Weight of the patient* The average drop in weight per week was 1 25 kg

4 *Albuminuria* Albuminuria persisted in spite

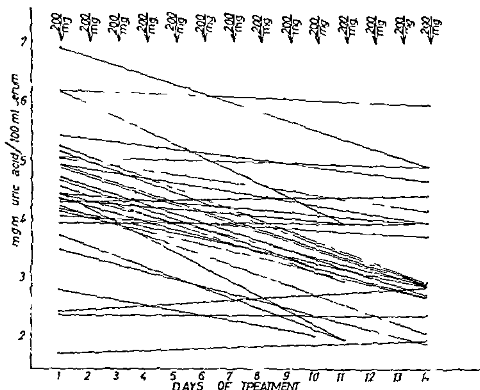



*Fig 6* Scattergram of the serum uric acid before and after natural progesterone therapy

Fig 6 demonstrates the reduction in serum uric acid levels during progesterone therapy

4 *Blood urea* In only 11 (27 5%) of the 40 patients with pre-eclamptic toxaemia the blood urea was reduced In the remaining cases the blood urea was unchanged

5 *Urea clearance* The urea clearance before treatment varied between 55 and 100% of the normal levels in 40 patients with pre-eclamptic toxaemia Two weeks after starting progesterone therapy improvement to above 75% of the normal values occurred in 22 patients (55%) no change appeared in 12 patients whose urea clearance before treatment was above 75% and in 6 patients the clearance dropped below 55% of normal

Fig 7 is a scattergram demonstrating the improvement in urea clearance in association with pre-eclamptic toxaemia under the effect of progesterone therapy

### *Effect of Natural Progesterone Administration on Normal Pregnancy*

Natural progesterone given over 2 weeks to 10 normal women at the 34th–38th week of pregnancy and in doses similar to those given to toxaemic patients did not produce any change in the blood pressure A diuresis of 20–50% of the mean 24 hour volume of urine prior to treatment was obtained in all cases and this was accompanied by a gain in weight less than the average in 6 cases

The serum sodium potassium and uric acid as well as the blood urea and urea clearance did not change

### *Obstetric management*

Labour was induced in all cases Delivery was unassisted in 23 cases extraction by forceps was required in 8 cases and by the vacuum extractor in 3 cases and lastly labour was terminated by caesarean section in 6 cases Only two fetal deaths occurred One of them was the outcome of fulminating pre-eclamptic toxaemia while in the 2nd case the mother was not responding to progesterone therapy and intrauterine fetal death was diagnosed 4 days before delivery

## DISCUSSION

It has been widely assumed that progesterone is a salt retaining hormone This assumption was

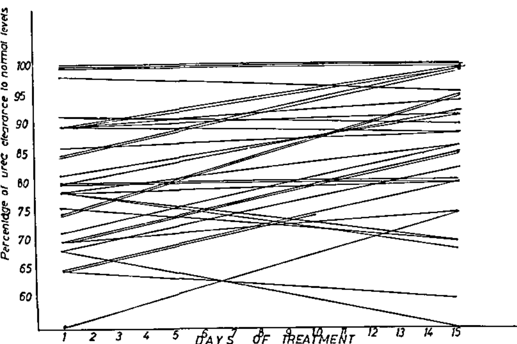



*Fig 7* Scattergram for the percentage of urea clearance to normal levels for toxaemic patients before and after natural progesterone therapy

favoured by the fact that progesterone administration could maintain the life of adrenalectomised animals (5 15) It also produced deoxycorticosterone like reduction of the urinary sodium–potassium ratio in adrenalectomised rats when given in large doses (9) These findings could be explained by the close structural similarity between progesterone and deoxycorticosterone the latter being a strong sodium retaining corticosteroid

However Landau et al (10 11) have shown clearly that progesterone had a natriuretic action which could not be elicited in adrenal deficient subjects unless aldosterone or deoxycorticosterone were administered Therefore they ascribed this natriuretic effect to inhibition of adrenal salt retaining hormones at a renal level Landau & Lugibihl (12) assumed that the natriuretic influence of progesterone in normal subjects is due to competitive inhibition of endogenous aldosterone Progesterone may be considered an aldosterone antagonist The authors showed that discontinuance of progesterone treatment caused sodium retention which might be due to compensatory oversecretion of aldosterone

Armstrong (1) first described the hypotensive action of progesterone in hypertensive rats dogs and human beings suffering from essential hypertension Genest (6) found that progesterone lowered the blood pressure of hypertensive patients Melby (14) found that when progesterone was administered to patients suffering from the syndrome of idiopathic oedema they experienced a diuresis with a high excretion of sodium and water within 24 hours after a single injection of 500 mg of 17 $\alpha$ hydroxyprogesterone caproate

The observations made in this work confirm the hypotensive and diuretic effect of progesterone in pre-eclamptic toxaemia It improves kidney function and it has no side effect either on the mother or the newborn In 80% of the cases studied it controlled the process of toxaemia with a well maintained hypotensive action None of our patients went into the convulsive state during progesterone medication No infant manifested any abnormality in the genital organs Our selection of controls was mainly based on the fact that we substituted progesterone which was rather deficient in pre-eclampsia as demonstrated in the vaginal smear pattern So the selection of normal pregnancy where the progesterone is within normal

values would offer a reasonable control Another way of looking at the problem is to select a control group treated with some other line of therapy not progesterone even rest in bed and the results are compared with those treated with progesterone Our findings would support the previous observations made by Dumitresco (3) who demonstrated a diuretic and a hypotensive effect of progesterone on pregnancy toxaemia

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*Submitted for publication Aug 1 1972*

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Acta Obstet Gynec Scand 54 203–208 1975

# RENOGRAPHIC EVALUATION OF RENAL EXCRETION IN HYDRONEPHROSIS OF PREGNANCY

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*Abstract* The functional significance of renographic delay in excretion observed in connection with chiefly unilateral physiologic hydronephrosis of pregnancy was analyzed in 10 cases The delay was found to be entirely or predominantly due to the reservoir effect of the dilated urinary tract A moderate reduction of urinary flow on the affected side was suggested to contribute to the impairment in only 3 of the 10 cases Interpretation of the degree of urinary obstruction as judged by renography should be cautious This applies especially to the assessment of complete obstruction which should be diagnosed only after considering the reservoir effect of the dilated tract

Since its introduction in 1956 (22) radio-isotope renography has been generally accepted as a simple means of estimating secretory (uptake) as well as excretory (drainage) function of the individual kidney (7 23) The safe application of this method during pregnancy with respect to the risk of radiation is satisfactorily established (17 19 25) and a few reports of the findings in normal pregnancy (3 12 16 18 19 20) and in pregnancy complicated by toxaemia (13 18) have been published During normal pregnancy a high incidence of impaired excretion has generally been reported Occurring as early as the twentieth week of gestation (3) the impairment develops with rising gestational age (3 19 20) and disappears rapidly following delivery (5 12 20) The condition is significantly more common and more pronounced on the right side (5 12 20) Posture influences the delay in excretion with partial relief when the patient changes from a supine to a lateral (3 16) sitting (18 19) or genupectoral (12) position

The impairment of excretion observed has generally been associated with the common occurrence of physiologic hydronephrosis during pregnancy (9) but a causal connection has not been established To some extent this seems to be due to lack of methodological standards for renography as well as of adequately defined parameters for interpretation Consequently severe impairments of excretion have sometimes been vaguely assessed in terms such as strongly suggestive of obstruction (20) indicative of relative or total obstruction (19) and in many patients compatible with complete obstruction (3) The significance of these conclusions may be questioned however When discussing time to the peak as a parameter of renal excretion the author (4) emphasized that renographically similar impairments of excretion can result from either raised renal tract dead space volume (reservoir effect) or lowered urinary flow rate or a combination of both This deficiency of renographic distinction requires analysis of either factor to clarify the functional significance of any impairment which does not seem to have been performed in the reports mentioned

In the present study the significance of renographic delay in excretion observed in connection with chiefly unilateral physiologic hydronephrosis of pregnancy was analyzed with respect to the prevailing conditions of dead space volume and urinary flow rate compared with the contralateral normal side The degree of hydronephrosis was estimated from urography pictures

## MATERIAL

Ten women were selected for the study when renography and a following intravenous urography had demonstrated chiefly unilateral moderate or severe impairment of renal excretion (Fig 1) associated with hydronephrosis and hydroureter predominantly on the

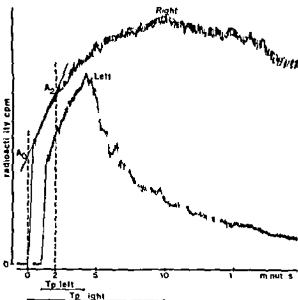



*Fig 1* Bilateral renograms showing a 4 3 times longer postrenal delay in excretion in association with right sided hydronephrosis of pregnancy (Patient I L Table 1) For the sake of clarity the left side renogram is displaced one minute in relation to zero time Right renal tract dead space volume was estimated to be only 2 4 times greater than the left (Fig 2) suggesting a relatively lowered urinary flow rate on the right side to contribute to the delay in excretion Time to the peak Tp is indicated for each renogram The constructions for determining uptake ratio are shown Uptake ratio was calculated as $A_2/A_0$.

ight side (Fig 2) In some cases a relatively slight latation was observed also in the left tract Nine of women were at various stages in the third trimester pregnancy whereas one was in the 26th week of pregnancy Four of the patients were nulliparae and the remaining six were primiparae

The women were initially submitted for routine renography because of obscure pain referable to the urinary tract In two cases the pain was left sided Patients with established urinary calculus toxaemia of pregnancy or a known history of chronic renal disease were not included in the series All of the patients had creatinine values of 1 0 mg/100 ml plasma or less

The interval between renography and intravenous urography generally did not exceed 3 days but in four cases the interval was 5 9 16 and 20 days respectively

Renography was repeated within 2 weeks following delivery in eight cases In two cases the interval was 3 weeks and 3 months respectively

## METHODS

*[¹³¹I] hippuran renography*

Two hours before examination the patients were given an oral dose of iodine solution equivalent to 700 mg iodide in order to block thyroid uptake of any free radioactive iodine Any medication was avoided on the day of examination except for 5–10 mg diazepam which was given in some cases The patients were hydrated with 800 ml of weak tea which was ingested over a period of 10–30 minutes beginning 30 to 60 minutes before the examination

Renography was performed with the patient comfortably seated The kidneys were located by means of available urography pictures or by screening with a hand-detector for maximal activity in the kidney regions after injection of 10 μCi [¹³¹I] hippuran

Two parallel 1¾×2 inch crystal scintillation detectors registered the ¹³¹I activity in the renal tracts separately Each detector had a conical collimator with an ellipsoid aperture and a length of 9 cm With the arrangements used the practical field of view in the plane of the kidney as determined to the line of 50% geometric efficiency was calculated to be 270×155 mm with circularly rounded short sides 0 3 μCi/kg body weight of [¹³¹I] hippuran was injected into an antecubital vein The tracer was diluted and given in less than 0 2 ml of fluid and the needle was immediately flushed with saline The renograms were recorded with a time constant of 3 seconds Paper speed was 10 mm/min and full range was calibrated to 30000 counts per minute

*Interpretation of the renogram*

For purposes of discussion and interpretation the renogram contour may be divided into three phases (7) The initial phase is the very rapid rise of the curve which corresponds to increasing background activity in the kid



*Fig 2* Intravenous urogram showing right sided hydronephrosis of pregnancy in the same patient as in Fig 1 (I L Table 1) Outlines of the left urinary tract are reinforced The volume of right dead space was estimated to be 2 4 times greater than the normal left

ney and adjacent tissues as well as to early parenchymal uptake of tracer. The following gradual rise towards the peak constitutes the second phase which is due to uptake and accumulation of test substance in the kidney and renal pelvis before it is evacuated by the urine from the field of detection. The slope of this phase may therefore be regarded as representing renal Hippuran uptake capacity. The renogram peak is followed by a declining third phase the slope of which chiefly reflects excretion of tracer to the bladder.

*Evaluation of renal excretion.* In the present investigation excretion of tracer was determined by the time to the renogram peak Tp which has been shown to be linearly correlated to the quotient between renal tract dead space volume $S$ and urinary flow rate $F$ (4)

$$Tp=0.86+k\,S/F \quad \text{where } k \text{ is a constant} \qquad [1]$$

The correlation given postulates that Hippuran uptake capacity is essentially normal. In principle the term 0.86 corresponds to intrarenal transit delay of tracer when $S=0$. Consequently the delay in excretion referable to postrenal conditions $S/F$ is determined as (Tp−0.86) min.

Unilateral relative delay in excretion can result from either raised dead space volume or lowered urinary flow rate or a combination of both as compared to the contralateral side. An expression for the correlation between a pair of kidneys in these respects may be obtained from equation [1]

$$\frac{Tp_R-0.86}{Tp_L-0.86} = \frac{Srel_R}{Srel_L} \Big/ \frac{F_R}{F_L} \qquad [2]$$

where $R$ and $L$ refer to the right and left kidney respectively. Tp is determined as the time elapsing from the renogram starting point until the curve reaches the maximum value. The time was estimated to the nearest half minute or one minute interval depending on the sharpness of the peak (Fig. 1). $Srel$ is an index of relative dead space volume as determined from an X ray urogram by the method described in this paper.

The correlation of equation [2] permits consideration as to what extent a side difference in excretion observed may be referable to a determined difference in dead space volumes $Srel_R/Srel_L$ and/or to a difference in bilateral urinary flow rates $F_R/F_L$ which may be approximately estimated (4). The correlation given postulates that Hippuran uptake capacity is essentially normal.

*Estimation of Hippuran uptake capacity.* The Hippuran uptake capacity of the individual kidney was established from the slope of the renogram uptake segment as determined by the uptake ratio (1). The slope was represented by the best tangent to the first 2 min of the uptake segment. The tangent was drawn to intersect with vertical lines from the time axis through the renogram starting point and a point 2 min later (Fig. 1). Uptake ratio was determined as the quotient between the fictive activities on the tangent at the min and the starting point intersects.

*Estimation of renal tract dead space volume*

As previously described in detail (4) a relative index of renal tract dead space volume within the field of detection was calculated for each kidney from the size of its frontal projection on an X ray urogram. When the ureter was partly invisible corresponding parts of the contralateral ureter were excluded. The outlines of the dead space were transferred to a clear acetate film and cut out. The true area of the dead space $A$ was calculated by weighing the piece and after correction for X ray magnification. An index of relative volume $Srel$ was calculated from the formula

$$Srel=(\sqrt{A})$$

The ratio between dead space volumes of a pair of kidneys was determined as $Srel_R/Srel_L$ where $R$ and $L$ refer to the right and left kidney respectively.

*Statistical analysis*

Student's $t$ test was used for comparison of uptake ratios.

## RESULTS

*Estimation of Hippuran uptake capacity*

Before the material was analyzed for renal excretion it was necessary to confirm the postulated normal Hippuran uptake capacity. No statistical difference ($p<0.05$) was found between bilateral uptake ratios. This was also true for a comparison with ipsilateral ratios of previously reported normal pregnant controls (4).

*Estimation of renal excretion*

The mean of Tp observed in the renograms of the hydronephrotic tract was found to be 11.8 min (range 8–17 min) whereas the mean of the contralateral normal side was 3.5 min (range 2–5 min) (Table I). The mean ratio between bilateral corrected Tp-values Tp−0.86 was found to be 4.8 indicating on average a nearly five times longer postrenal delay in excretion on the dilated side.

Following delivery excretion as determined by renography became normal in 6 cases (time to the renogram peak ≤3 min) (4). A slight delay (Tp 3.5–4 min) remained in three cases. In one case the examination was technically insufficient.

*Ratio between bilateral dead space volumes*

When the $Srel$ values of the dilated sides were compared to those of the contralateral normal a mean ratio of 4.0 (range 2.4–6.5) was found (Table I) indicating that the hydronephrotic tract on average was four times larger than the contralateral normal. However there was a great range for $Srel$ values on both sides. In 8 cases

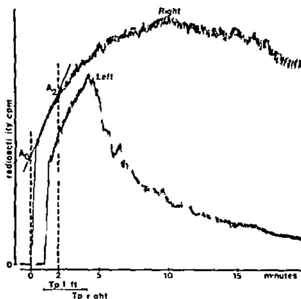



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The interval between renography and intravenous urography generally did not exceed 3 days but in four cases the interval was 5 9 16 and 20 days respectively

Renography was repeated within 2 weeks following delivery in eight cases In two cases the interval was 3 weeks and 3 months respectively

## METHODS

### [131] *hippuran renography*

Two hours before examination the patients were given an oral dose of iodine solution equivalent to 200 mg iodide in order to block thyroid uptake of any free radioactive iodine Any medication was avoided on the day of examination except for 5–10 mg diazepam which was given in some cases The patients were hydrated with 800 ml of weak tea which was ingested over a period of 10–30 minutes beginning 30 to 60 minutes before the examination

Renography was performed with the patient comfortably seated The kidneys were located by means of available urography pictures or by screening with a hand-detector for maximal activity in the kidney regions, after injection of 10 μCi [125I] hippuran

Two parallel 1¾×2 inch crystal scintillation detectors registered the 131I activity in the renal tracts separately Each detector had a conical collimator with an ellipsoid aperture and a length of 9 cm With the arrangements used the practical field of view in the plane of the kidney as determined to the line of 50% geometric efficiency was calculated to be 220×155 mm with circularly rounded short sides 0 3 μCi/kg body weight of [131I] hippuran was injected into an antecubital vein The tracer was diluted and given in less than 0 2 ml of fluid and the needle was immediately flushed with saline The renograms were recorded with a time constant of 3 seconds Paper speed was 10 mm/min and full range was calibrated to 30000 counts per minute

### *Interpretation of the renogram*

For purposes of discussion and interpretation the renogram contour may be divided into three phases (7) The initial phase is the very rapid rise of the curve which corresponds to increasing background activity in the kid



*Fig 2* Intravenous urogram showing right sided hydronephrosis of pregnancy in the same patient as in Fig 1 (I L Table I) Outlines of the left urinary tract are reinforced The volume of right dead space was estimated to be 2 4 times greater than the normal left

ment of impaired excretion requires consideration of the reservoir effect of the dilated urinary tract for proper estimation of urinary flow conditions

In the present investigation the functional significance of renographic delay in excretion was analyzed in 10 pregnant women with urographically established hydronephrosis entirely or predominantly on the right side The physiologic nature of the condition was suggested by the return to normal excretion following delivery In one case with technically insufficient renograms intravenous urography revealed marked regression of dilatation

As the renographic picture of excretion is considered to be influenced also by impaired Hippuran uptake capacity (4 10) it became necessary to establish the prevailing situation in this respect Analysis of the renogram uptake ratios indicated that uptake capacity was essentially normal in the cases studied

The bilateral renograms obtained during pregnancy indicated on average a nearly five times longer postrenal delay in excretion on the dilated right side Despite promotion of excretion by hydration (4 26) and the sitting position of the patient (18 19 ?6) the delay on the right side may be considered severe (Tp >12 min) in four of the cases

The volume of the dilated right urinary tracts was estimated to be on average four times greater than the normal left Incomplete estimations of relative renal tract dead space volume Srel in two cases were not considered to influence appreciably the ratios between bilateral volumes The absolute values of Srel in the remaining eight cases showed a great range on both sides with a mean of 287 on the right side and 65 on the left For comparison it is of interest that in 20 non-pregnant women the mean of Srel was found to be 44 (range ?4–75) (6) indicating that dead space volume of the present series on average was enlarged six and a half times on the right side and one and a half times on the left

In seven cases the ratios of bilateral dead space volumes (Srel right/left Table I) approximate or exceed corresponding ratios of excretion (Tp ~0 86 right/left Table I) indicating that the side differences in excretion observed may be entirely or predominantly explained by the side differences in dead space volumes As a matter of fact the expected difference in excretion in two patients (E A and U S Table I) was not attained suggesting a higher urinary flow rate on the dilated side compared with the contralateral normal side

In three cases (I L M B K U Table I) the observed difference in excretion was not fully explained by the differing dead space volumes which suggests that a relatively lower urinary flow rate on the affected side contributed to the delay The possibility of intrarenal delay ( parenchymal stasis ) (21) was considered unlikely in the present cases with normal uptake function

The physiologic significance of the side difference of urinary flow indicated in some cases cannot be decisively assessed because the error of the estimation employed is not established However in four of the cases studied simultaneous direct measurements of bilateral urinary flow rate as reported elsewhere (4) resulted in flow ratios (D right/left Table I) in reasonable accord with the values estimated As a suggestion the side difference of urinary flow may reflect intermittent ureteric emptying on the dilated side as reported by Baird (2) and Gremme (14) Accepting a difference of flow amounting to 20 per cent as normal in non obstructed kidneys (8) the greatest estimated difference of 50% may be considered as quite moderate

From the present investigation it is concluded that the renographic delay in excretion observed in association with physiologic hydronephrosis of pregnancy is entirely or predominantly due to the reservoir effect of the dilated urinary tract In some cases a moderate reduction of urinary flow on the affected side may contribute to the delay Interpretation of the functional aspects of impaired excretion as judged by renography should be cautious This applies specially to the assessment of complete obstruction Preferably excretion is promoted during the test by hydration and the sitting position of the patient It is emphasized that proper interpretation of impaired excretion requires consideration of the reservoir effect of a dilated urinary tract

## ACKNOWLEDGEMENTS

Renography was performed at the Radiation Physics Department Sahlgren Hospital where valuable assistance was given by Mrs Ulla Knute The X ray examinations were made at the Roentgendiagnostic Department II Sahlgren Hospital by Sture Stattin M D and Magnus

Rosencrantz M D to whom I am very grateful Statistics were scrutinized by research assistant Anders Lundberg University of Gothenburg

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*Submitted for publication Jan 22 1974*

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Acta Obstet Gynec Scand 54 209–211 1975

# PHYSIOLOGICAL ROLE OF MECONIUM DURING DELIVERY

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*Abstract* Women with green amniotic fluid ($N=495$) delivered more quickly after membrane rupture (mean 2 7 h) than 495 controls with normal-coloured amniotic fluid (mean 4 6 h) whereas the labour in the first group lasted longer prior to membrane rupture This difference was not caused by active treatment difference in the gestational age or size of the infants In the light of our previous findings of immunoreactive oxytocin in meconium our results may suggest that meconium serves as a fetal reservoir of biologically active compounds emptied into the amniotic fluid to expedite the termination of labour in fetal distress

Recent evidence indicates that the fetal posterior pituitary may be involved in the release of oxytocin during human labour (1) and fetal oxytocin may reach the uterus through fetal urine and amniotic fluid (3) Using radioimmunoassay we found large amounts of immunoreactive oxytocin in meconium and green amniotic fluid (3) The biological significance of this material is unknown although meconium-contaminated green amniotic fluid is often associated with fetal distress We studied the association of green amniotic fluid with the duration of human labour and found that green amniotic fluid might play a physiological role during delivery

## MATERIALS AND METHODS

The duration of labour was recorded in 990 parturients 495 consecutive women with green amniotic fluid at the time of membrane rupture belonged to the test group The controls were 495 parturients of the same parity successive to each proband Patients who had received oxytocic agents or had any operative manoeuvres interfering with the spontaneous course of delivery were not included in either group The test group and the controls were similar as to the mean duration of gestation the mean birth weight and length and the mean head circumference

## RESULTS

The total mean duration of labour was similar in the test group and the controls (Fig 1) In women whose amniotic fluid was green at the time of membrane rupture the mean interval between membrane rupture and delivery was significantly shorter than in the controls (Table I) while the mean interval between membrane rupture was longer in women with green amniotic fluid than in the controls The difference was statistically significant in both primiparous and multiparous groups (Table II)

## DISCUSSION

A critical obstetric question is whether green amniotic fluid plays a physiological role during deliv

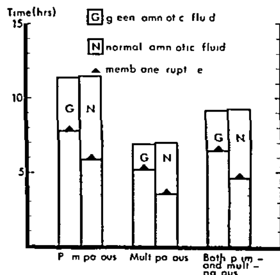



*Fig 1* The mean duration of labour in relation to membrane rupture The hatched areas represent intervals between the onset of labour and membrane rupture

Acta Obstet Gynec Scand 54 213–216 1975

# SERUM LIPIDS DURING OESTRADIOL VALERATE/NORGESTREL TREATMENT OF MENOPAUSAL WOMEN

*Double blind Study of a Sequential Preparation*

Niels Chr Christensen Paul Chr Davidsen Niels J Secher and Gunnar Thomsen Pedersen

*From the Department of Obstetrics and Gynaecology (Heads J J Jacobsen K Kristoffersen and G Thomsen Pedersen) University Hospital of Odense Odense Denmark*

*Abstract* Thirty three women aged 38–56 years all with menopausal complaints were treated after randomization during three cycles either with oestradiol valerate/norgestrel sequential therapy or a placebo and then during three cycles with the opposite drug Serum cholesterol triglycerides glycerol as well as plasma FFA and lipoprotein electrophoresis were studied before the institution of treatment after each cycle and 4 weeks after the intake of the last tablet During the treatment period the triglyceride level was significantly lower than during the placebo period whereas no changes occurred in other parameters The decrease was at a maximum after 8 weeks treatment 4 weeks after the discontinuation of treatment the levels were as before its institution It cannot be decided whether the decrease was due to the natural oestrogen or to the gestagen

Serum lipid variations during treatment with combined oestrogen/gestagen preparations have been the subject of a number of investigations showing consistently an increase in the concentration of triglycerides during the medication (8 10 12 14) These studies have been concerned almost exclusively with fertile women receiving synthetic oestrogen/gestagen for contraceptive purposes

Such agents have also been used in the treatment of women having menopausal complaints but it is uncertain whether such women are likely to exhibit similar changes in lipid metabolism during the medication Owing to the changes in endogenous hormone production during the menopause it is not possible to transfer the results directly from the younger women

Comparisons of various combination type and sequential preparations are difficult This is not only because of the different content of oestrogen and gestagen but the various synthetic hormones may also exert different effects upon the serum lipids In addition this effect may differ from that of the natural hormones

We therefore investigated serum cholesterol triglycerides glycerol plasma free fatty acids (FFA) and lipoprotein electrophoresis in 33 women treated for menopausal complaints with a sequential preparation containing natural oestrogen and gestagen This investigation was carried through as a double blind study in which the lipid concentrations on hormone therapy during three cycles were compared with the levels during a corresponding placebo period

## MATERIAL AND METHODS

The series consist of 33 women aged 38–56 years (mean age 47) All had menopausal complaints such as irregular bleeding (31 patients) and/or vasomotor symptoms (76 patients)

Apart from that none had any symptoms or signs of disease before or during the treatment

The investigation was performed as a double-blind study using Cycloprogynova® and placebo Cycloprogynova is a sequential preparation containing 2 mg oestradiol valerate per tablet and the last 10 tablets also 0.5 mg (d l) norgestrel The dosage was one tablet daily for 21 days followed by an interval of 7 days According to randomization the patients were treated with active drug or placebo during 3 cycles and then during another 3 cycles with the other preparation

The patients attended the Out patient Department at the institution of the trial returning on the day after taking the last tablet in each cycle and again 4 weeks

Table I *Mean values (x) standard deviation (S D ) and range of lipid concentrations in serum of 33 women on oestradiol valerate/norgestrel and placebo medication*

Values stated are in mM/l

| | Cyclo-progynova | Placebo | *Significance of difference between means* |
|---|---|---|---|
| Cholesterol | | | |
| *x* | 6 6 | 6 9 | *p*>0 05 |
| S D | 1 3 | 1 2 | |
| Range | 4 9–9 4 | 5 0–10 1 | |
| Triglycerides | | | |
| *x* | 0 84 | 1 20 | *p*<0 001 |
| S D | 0 21 | 0 36 | |
| Range | 0 48–1 37 | 0 57–2 07 | |
| Glycerol | | | |
| *x* | 0 06 | 0 07 | *p*>0 05 |
| S D | 0 02 | 0 02 | |
| Range | 0 03–0 10 | 0 03–0 14 | |
| Free fatty acids | | | |
| *x* | 0 39 | 0 42 | *p*>0 05 |
| S D | 0 12 | 0 15 | |
| Range | 0 10–0 51 | 0 20–1 03 | |

after the medication had been discontinued They were fasting and venous blood was drawn for determination of serum cholesterol triglycerides and glycerol as well as plasma FFA and lipoprotein electrophoresis The *es were stored on ice until centrifuged within* hour Samples which were not analysed at once were at −20°C except for plasma for the lipoprotein lectrophoresis which was stored at 4 C

Analytical methods Cholesterol Huang et al (6) (coefficient of variation 5%) triglycerides and glycerol Eggstein & Kreutz (3) (coefficient of variation 5%) FFA Laurell & Tibbling (9) (standard deviation 3 mM/L) and lipoprotein electrophoresis on paper according to Hatch & Lees (5)

For 20 patients all the lipid values are available whereas for the others the values from one or two of the test days are missing for various technical reasons

The patients were weighed after the 3rd and after the 6th cycle The difference exceeded two kg in only 4 of the patients 3 of whom weighed more after treatment with hormone and one more after placebo

## RESULTS

Table I gives the *mean concentrations of serum* cholesterol triglycerides and glycerol as well as plasma FFA during the treatment period and placebo period It shows a significantly and markedly lower triglyceride level during the treatment period than during the placebo period whereas the slight changes in cholesterol glycerol and FFA are not significant In 31 of the 33 patients the mean value of the triglyceride concentrations was lower during the treatment period than during the placebo period while this applied to cholesterol in only 19 of the cases

The result of lipoprotein electrophoresis did not show chylomicra in any case Therefore the change in triglyceride concentration was presumably due to a change in the concentration of pre beta lipoprotein Pre-beta lipoprotein was found in 12 of the patients at one or more tests

*Although practically all* the patients showed lowered triglyceride levels during the treatment period the decrease was most marked in those who exhibited the highest values during the placebo period Thus a correlation analysis revealed a positive correlation between the difference in mean value during the placebo and treatment period and the mean value during the placebo period ($r$=0 84 $p$<0 01)

The mean value *of the triglyceride concentration* after 4 weeks treatment with Cycloprogynova was 0 95 mM/l after 8 weeks treatment 0 82 mM/l and after 12 weeks 0 83 mM/l The difference *between the levels after* 4 and 8 weeks treatment was significant when assessed by a paired $t$ test ($p$<0 01) Thus it took more than 4 weeks *for the change in triglyceride concentration to be*come maximal whereas no further decrease occurred after 8 weeks treatment

The mean serum triglyceride level before treatment was 1 26 mM/l The mean value 4 weeks after the hormone medication had been discontinued (18 were at that time on placebo) was 1 ?? mM/l *In the 15 subjects for whom samples were* available 4 weeks as well as 8 weeks after the discontinuation of hormone therapy the mean values were 1 22 and 1 33 mM/l respectively This difference is not significant From Table I it is apparent that the mean value on placebo was 1 ?0 mM/l which corresponds to the pre treatment level and to the level 4 weeks *after discontinuation* of Cycloprogynova

To assess whether it made any difference *whether placebo or* Cycloprogynova was administered first the mean value of the triglyceride concentration during the placebo period was calculated for those subjects who received Cycloprogynoca first 1 21 mM/l *and those who received* placebo first 1 16 mM/l

## DISCUSSION

The present finding of a decrease in serum triglycerides on treatment with a combined oestrogen/gestagen preparation is in contrast to investigations which have shown an increase in triglycerides in women receiving these hormones for contraceptive purposes (8 10 12 14) This disparity may be due to differences in the selection of the patients or to differences in the preparations used Our patients were older than those of previous series they had menopausal complaints we used a natural oestrogen and a relatively high concentration of gestagen

The serum triglyceride concentration rises in women with advancing age but considerably less so than in men (1) This difference applies also to cholesterol and has led to presumptions about a lipid lowering effect of the ovarian hormones However oophorectomy in premenopausal women does not appear to lead to any increase in serum cholesterol (13) The patients of the present series were selected because they had menopausal complaints and therefore their ovarian hormone production may be imagined to have been particularly low They also had relatively high triglyceride levels compared with a selected normal series in which we found a mean level of 0 98 mM/l in women aged 40–59 (1) However the great majority of patients in the present study had levels within the the normal range and as no regard was paid to body weight the patients are not directly comparable with those of the other study Moreover the triglyceride levels decrease also in patients with low concentrations but not as much as in those with high concentrations Therefore it does not seem likely that the selection of the patients is the explanation of the decrease in triglycerides

The increase in triglycerides found by others on combined preparations containing synthetic oestrogen and gestagen has been ascribed to the oestrogen component Indeed such an increase has also been observed on administration of pure synthetic oestrogen (12) There have been a few investigations into the serum lipid variations in postmenopausal women on oestrogen medication Florian et al (4) studying patients on synthetic oestrogen found no changes in the cholesterol or triglyceride levels Utian (13) using oestradiol valerate and Robinson et al (11) using equine oestrogen observed a decrease in the serum cholesterol concentration Neither determined the triglycerides Robinson et al (11) moreover found the magnitude of the fall in cholesterol level to be dose-dependent

The effect of gestagens upon the serum lipids has been less extensively investigated than that of synthetic oestrogens It applies to the gestagens even more so than to the oestrogens that differences in effect must vary from drug to drug depending for example upon the degree of antioestrogenic and androgenic action Larsson-Cohn et al (8) found a slight increase in serum triglycerides on norethindrone whereas no such effect was found upon cholesterol Spellacy et al (12) on the other hand found a minor decrease in triglycerides in women treated *post partum* with ethynodiol acetate Since however this decrease did not differ from that in patients who had had I U D inserted it was not interpreted as a consequence of gestagenic action

In a study using—for contraception—the same gestagen as in our investigation Eckstein et al (2) found a significant decrease in the cholesterol level in the course of the treatment. The triglycerides were not determined In a series investigated also for triglyceride kinetics on treatment with oestrogens and gestagens separately and combined Kissebah et al (7) found a lower triglyceride level on gestagen treatment than in control subjects Since the clearance of exogenous as well as endogenous triglycerides proved elevated on gestagen medication this was assumed to have caused the lower level This investigation showed no change in FFA

The possibility that the lowered triglyceride concentration in the present study too may have been due to an increased output from the blood and not to a reduced production or increased breakdown accords with the unchanged FFA and glycerol levels which demonstrated unchanged lipolytic activity

Thus genuine oestrogen as well as gestagen appears to be able to depress the serum lipid concentration The present investigation does not permit conclusions as to whether the decrease in triglycerides found by us was due to one of the components and which one or to the combination of both

Table I *Mean values (x) standard deviation (S D) and range of lipid concentrations in serum of 33 women on oestradiol valerate/norgestrel and placebo medication*

Values stated are in mM/l

| | Cyclo-progynova | Placebo | Significance of difference between means |
|---|---|---|---|
| Cholesterol | | | |
| $x$ | 6 6 | 6 9 | $p>0\ 05$ |
| S D | 1 3 | 1 2 | |
| Range | 4 9–9 4 | 5 0–10 ? | |
| Triglycerides | | | |
| $x$ | 0 84 | 1 20 | $p<0\ 001$ |
| S D | 0 21 | 0 36 | |
| Range | 0 48–1 37 | 0 57–2 07 | |
| Glycerol | | | |
| $x$ | 0 06 | 0 07 | $p>0\ 05$ |
| S D | 0 02 | 0 02 | |
| Range | 0 03–0 10 | 0 03–0 14 | |
| Free fatty acids | | | |
| $x$ | 0 39 | 0 42 | $p>0\ 05$ |
| S D | 0 12 | 0 15 | |
| Range | 0 10–0 51 | 0 20–1 03 | |

after the medication had been discontinued They were fasting and venous blood was drawn for determination of serum cholesterol triglycerides and glycerol as well as plasma FFA and lipoprotein electrophoresis The ples were stored on ice until centrifuged within hour Samples which were not analysed at once were red at −20°C except for plasma for the lipoprotein lectrophoresis which was stored at 4 C

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For 20 patients all the lipid values are available whereas for the others the values from one or two of the test days are missing for various technical reasons

The patients were weighed after the 3rd and after the 6th cycle The difference exceeded two kg in only 4 of the patients 3 of whom weighed more after treatment with hormone and one more after placebo

## RESULTS

Table I gives the mean concentrations of serum cholesterol triglycerides and glycerol as well as plasma FFA during the treatment period and placebo period It shows a significantly and markedly lower triglyceride level during the treatment period than during the placebo period whereas the slight changes in cholesterol glycerol and FFA are not significant In 31 of the 33 patients the mean value of the triglyceride concentrations was lower during the treatment period than during the placebo period while this applied to cholesterol in only 19 of the cases

The result of lipoprotein electrophoresis did not show chylomicra in any case Therefore the change in triglyceride concentration was presumably due to a change in the concentration of pre beta lipoprotein Pre beta lipoprotein was found in 12 of the patients at one or more tests

Although practically all the patients showed lowered triglyceride levels during the treatment period the decrease was most marked in those who exhibited the highest values during the placebo period Thus a correlation analysis revealed a positive correlation between the difference in mean value during the placebo and treatment period and the mean value during the placebo period ($r=0\ 84$ $p<0\ 01$)

The mean value of the triglyceride concentration after 4 weeks treatment with Cycloprogynova was 0 95 mM/l after 8 weeks treatment 0 82 mM/l and after 12 weeks 0 83 mM/l The difference between the levels after 4 and 8 weeks treatment was significant when assessed by a paired $t$ test ($p<0\ 01$) Thus it took more than 4 weeks for the change in triglyceride concentration to become maximal whereas no further decrease occurred after 8 weeks treatment

The mean serum triglyceride level before treatment was 1 26 mM/l The mean value 4 weeks after the hormone medication had been discontinued (18 were at that time on placebo) was 1 2? mM/l In the 15 subjects for whom samples were available 4 weeks as well as 8 weeks after the discontinuation of hormone therapy the mean values were 1 22 and 1 33 mM/l respectively This difference is not significant From Table I it is apparent that the mean value on placebo was 1 20 mM/l which corresponds to the pre treatment level and to the level 4 weeks after discontinuation of Cycloprogynova

To assess whether it made any difference whether placebo or Cycloprogynova was administered first the mean value of the triglyceride concentration during the placebo period was calculated for those subjects who received Cycloprogynoca first 1 21 mM/l and those who received placebo first 1 16 mM/l

Acta Obstet Gynec Scand 54 217–221 1975

# PLASMA LEVELS OF PROGESTERONE AND OESTROGENS IN WOMEN TREATED WITH AN ORAL CONTRACEPTIVE OF LOW OESTROGEN CONTENT (OVOSTAT 1375)

Elof D B Johansson

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*Abstract* The plasma levels of progesterone and oestrogens were measured during treatment with an oral con traceptive containing 37 5 μg of ethinyl oestradiol and 1 mg of lynestrenol (= 3-desoxy 17α-ethinyl 19 nor testosterone) (Ovostat 1375 Organon Oss Holland) Blood samples were taken every other day during 9 com plete cycles in 5 healthy women The plasma levels of progesterone were in the range of those found during the early follicular phase of the normal women No ovula tion seemed to have occurred The mean plasma levels of oestrogens (oestradiol and oestrone) during treatment were below those found during the early follicular phase of 34 normal cycles although overlapping of values occurred The ratio between oestradiol and oestrone during treat ment was 1 2 vs 1 1 during the early follicular phase of the normal cycles In two cycles treatment was started during the midcyclic rise of oestrogens No rise of proges terone indicating corpus luteum formation was found

Despite the low amount of oestrogen in the drug the ova ries appeared to secrete minimal amounts of sex steroids The lynestrenol content is likely to be partly responsible for the inhibition of ovulation and the additional suppres sion of steroidogenesis

The oestrogen component in the combined oral contraceptive pills appears to be a major contribu tor to the side effects associated with this medica tion This appears to be especially true for the elevation of plasma lipids (13) and changes in glu cose metabolism (15) while the relation to throm boembolism is still a matter of dispute (6) There fore efforts are made to reduce the amount of oestrogen in the oral contraception but still main tain inhibition of ovulation

The purpose of the present study was to in vestigate the effect on the ovarian function of 37 5 μg of ethinyl oestradiol combined with 1 mg of lynestrenol

## MATERIAL AND METHODS

Five young and healthy women volunteered to take part in this study The volunteers were accustomed to venopunctures during previous studies (7 10)

Venous blood samples were obtained daily during one control cycle and every other day during three con secutive cycles in five women treated with a combined oral contraceptive containing 37 5 μg of ethinyloes tradiol and 1 mg of lynestrenol (Ovostat 1375 supplied by N V Organon Oss Holland) One tablet was taken every day from the first day of bleeding for 22 days followed by 6 tablet free days This treatment was given during 16 cycles During th- Christmas and New Year holiday it was impossible to adhere strictly to the venopuncture schedule However enough samples were obtained to determine that no ovulation occurred in these cycles Due to persistent pelvic pains one subject (KÖ Table I) dropped out the study pre maturely In the remaining 9 cycles the blood samples were taken according to the plan described above

### *Assay methods*

Plasma oestrogens were measured by radioimmuno-assay using an antiserum prepared by Férin et al (1968) The assay technique followed the method de scribed by Hotchkiss et al (5) with minor modifications (3) The antiserum binds oestradiol 17β with high affinity but there is a 30% reaction with oestrone In a separated study oestradiol and oestrone were sepa rated on Sephadex LH 20 columns These analyses were made on samples from this study and also on samples obtained during treatment with 4 other types of oral contraceptives The ratio between oestradiol and oestrone was found to be 1 2 during the treatment with the oral contraceptives as compared to a ratio of 1 1 during the early follicular phase of normal cycles (11) No separation between oestradiol and oestrone was made in this study which means that the oestrone content of plasma will contribute close to 50% of the reading reported here No interference from oestriol or ethinyl oestradiol was found in the ranges used The detection limit for oestradiol in the radioimm

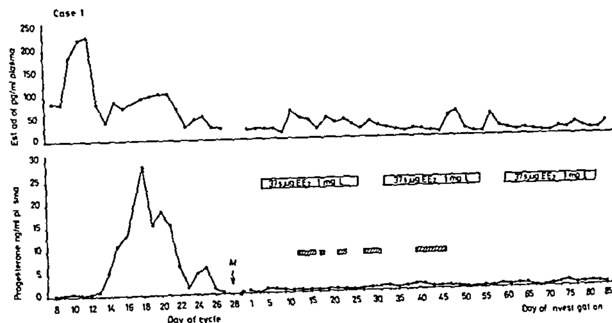



*Fig 1* Subject 1 Plasma levels of progesterone and oestrogen during one control cycle followed by three 22-day-cycles of treatment with Ovostat 1375 (KE) Vaginal bleeding is indicated by shaded areas L= lynestrenol

system used was 5 pg As 0 4 ml of plasma was used for extraction values between 10 and 15 pg/ml were at the detection limit of the assay system

The plasma levels of progesterone were measured by the rapid competitive protein binding technique de scribed by Johansson (7 8) Progesterone was also measured by radioimmunoassay (16)

Table I *Initials age number of previous preg nancies in the women treated with Ovostat 1375 together with treatment cycles and side effects*

| Sub-ject | Age | Previous preg nancies | No of treat ment cycles | Side effects |
|---|---|---|---|---|
| KE | 23 | 0 | 4 | Irregular bleeding amenorrhea pelvic pains |
| UG | 24 | 1 | 3 | Irregular bleeding |
| BL | 76 | 1 | 3 | Irregular bleeding |
| ML | 25 | 1 | 3 | None |
| KÖ | 21 | 0 | 3 | Irregular bleeding pelvic pains |

## RESULTS

The clinical side effects associated with the treat ment with Ovostat 1375 are summarized in Table I Irregular bleeding generally occurred only in the first treatment cycle Subject I who had amenorrhoea after the first treatment cycle had anovulatory cycles for four months after she stopped treatment The other four women resumed regular ovulatory cycles promptly after the end of treatment

The plasma levels of oestrogen and progesterone during treatment are illustrated in Fig 1 No ovula tions occurred during treatment as judged by the very low levels of progesterone in plasma and the absence of ovulatory peaks of the oestrogens In order to give a better illustration of the differ ence between the oestrogen and the progesterone levels during treatment as compared to those found during normal ovulatory menstrual cycles the oestrogen and progesterone levels measured during the 9 complete treated cycles have been summarized and compared with those previously found during 34 normal ovulatory cycles The total oestrogen levels are shown in Fig 2 The total range and mean levels are shown for the 9 treat ment cycles while the mean and 95% confidence

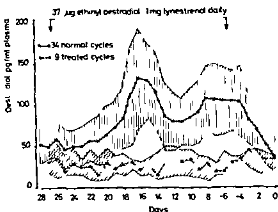



*Fig 2* Plasma levels of oestrogens during 34 normal cycles as compared to 9 cycles treated with Ovostat 1375 The cycles were synchronized and analysed as described in the legend to Fig 3

limits are shown for the 34 normal cycles The progesterone levels are shown in Fig 3 Very small fluctuations in oestrogen and progesterone levels occurred during treatment The mean oestrogen levels during treatment were lower than the early follicular phase levels during the normal cycles but overlapping of values occurred On several days the levels were at the dectection lim

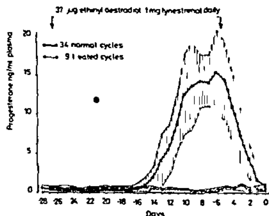



*Fig 3* Plasma levels of progesterone during 34 normal cycles as compared to 9 cycles treated with Ovostat 1375 In the normal cycles the first day of the following menstrual bleeding was called day 0 The Ovostat 1375 tablets were taken daily between days 27 and day -6 For the normal cycles means and 95% confidence limits are given and for the treated cycles means and total range

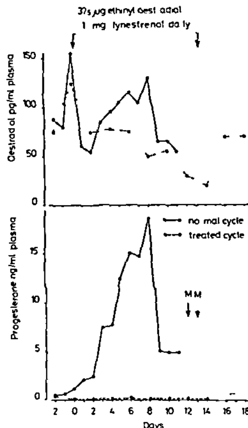



*Fig 4* Plasma levels of progesterone and oestrogen during one cycle during which Ovostat 1375 was given from the day of the midcycle peak of oestrogen and onwards A previous untreated cycle from the same woman is superimposed

its for the assays This occurred most frequently in the progesterone assay When these samples were reassayed by radioimmunoassay no significant difference was found compared to the early follicular phase of the untreated cycles

In order to study how late in the cycle Ovostat 1375 could be given and still inhibit ovulation a preliminary study was done during 2 cycles where the treatment was started when the oestrogen levels had started to increase One of these cycles is shown in Fig 4 The first tablet was given at day 12 in the cycle when the oestrogen levels were relatively high The treatment was continued for 14 days The oestrogen levels decreased during treatment to levels similar to those during the early follicular phase and no rise in plasma levels of progesterone was seen indicating lack of

a functioning corpus luteum (Fig 4) In the sec ond these two cycles the results were similar to those shown in Fig. 4

## DISCUSSION

The development of sensitive and rapid methods for determination of progesterone and oestrogen in plasma has recently made it possible to study the endogenous peripheral plasma levels of these hor mones during treatment with oral contraceptives As shown in another report from this laboratory (11) the oestradiol levels during treatment with combined oral contraceptives are lower than what is usually found during the earlier part of the follicular phase of the cycle but are higher than those observed in postmenopausal women

The oestrone levels were found to be reduced to a lesser extent These findings are at variance with previous reports (2 14) where no difference in oestradiol levels were observed during treat ment with oral contraceptives and the early fol licular phase of the normal cycle

The low levels of oestrogen and progesterone found in this study could probably not be ex plained entirely by the inhibition of ovulation The levels of FSH and LH during treatment with oral contraceptives appear to fall in the same range as found in the follicular phase of the normal cycle Accordingly early follicular phase levels of ovarian steroids would be expected during treat ment with oral contraceptives The additional reduction of the ovarian steroid levels apart from that caused by the lack of ovulation might be ex plained by a direct inhibition of steroid production in the ovary by the gestagenic part of the pill (10) Lynestrenol alone in high dosages has been found to suppress both progesterone and oestrogen levels (Johansson 1973)

One of the most important findings in this study is that suppression of ovulation can be achieved with a formulation containing an amount of oestrogen that is unlikely to inhibit ovulation but combined with an amount of gestagen that is high enough to inhibit ovulation The low levels of endogenous oestrogen during treatment would mean that the oestrogen content of the tablet is responsible for the major oestrogenic effects in the body As the endogenous oestrogen levels seem to be the same in women treated with oral contraceptives containing high amounts of oestrogen as in those women treated with oral contraceptives containing low amounts of oestrogen (11) the net effect on the target organs would be a reduction of oestrogen effect in the women treated with oral contraceptives containing low amounts of oestrogen It is therefore likely that oral contraceptives containing low amounts of oestrogen would show a lower frequency of those metabolic side effects associated with oestrogen However lack of adequate bleeding control may exclude these formulations from widespread clini cal use

## ACKNOWLEDGEMENTS

This study was supported by Organon Oss Svenska AB The author is grateful to the skilled technical staff at the Primate Laboratory for Research in Reproduction and to the volunteers for their dedication and endur ance

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*Submitted for publication Jan 29 1974*

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Acta Obstet Gynec Scand 54 223–226 1975

# ELEVATED LYMPHOCYTE ATP ase ACTIVITY IN PATIENTS WITH CANCER OF THE UTERINE CERVIX

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*Abstract* Elevated lymphocyte Adenosine Tri Phos phatase (ATP-ase) activity was found in 23 och 28 patients with cervical uterine carcinomas of various stages Treatment with external irradiation and radium insertion was followed by a decline in the lymphocyte ATP-ase activity in 22 patients while the activity remained unchanged in one patient No correlation between the tumour stage and the lymphocyte ATP ase activity was demonstrated In 24 patients the clinical effect of the treatment was well correlated with the decline in ATP ase activity It is suggested that the determination of lymphocyte ATP ase activity could be valuable in screening for cervical carcinoma and for follow-up after radical treatment

The important role of the circulating thymus derived lymphocyte in host resistance against the growth of neoplastic cells has long been known and extensively reviewed (10) Adherence of the lymphocyte to surface antigenic sites of the cancer cell probably after previous sensitization by tumour associated soluble antigens could initiate an intracellular sequence of events beginning with activation of the membrane bound (Na K) ATP ase followed by a nuclear activation with subsequent blast transformation of the lymphocytes (2) Turning into killer cells by these mechanisms the thymus derived lymphocytes would be able to destroy the malignant cell by the production and secretion of lymphokines (9) provided blocking antibodies do not arise which neutralise the cytotoxic effect by forming complexes with tumour antigens (1 11) Thymus derived lymphocytes in the peripheral blood seem to maintain their cytotoxicity against growing malignant tissue whereas lymphocytes within malignant tumours and their regional lymph nodes develop anergy against the tumor cells (4 15 16) probably due to an immunological paralysis after prolonged exposure to soluble tumour antigens (1)

On the theoretical basis that such activities of the circulating lymphocytes require energy examinations of the ATP ase activity of lymphocytes were undertaken and in previous experiments (3 5 6) it was demonstrated that the ATP ase activity of circulating lymphocytes was significantly higher in various forms of human malignant disease as compared with appropriate normal controls The lymphocyte ATP ase activity was characterized as a mitochondrial ATP ase activity primarily because it was independent of the sodium and potassium concentration of the assay was increased by addition of 2 4 dinitrophenol and was inhibited by oligomycin Furthermore the presence of functioning mitochondria in the assay mixture was demonstrated

Proceeding on the basis of the above considerations and previous results the present study was undertaken in order to extend the examination of lymphocyte activity to malignant disease of the female genital tract In addition it was necessary to have better reference material after improvements in the technique Finally it was of considerable interest to see how the lymphocyte ATP ase activity reacted to radical treatment of the patients genital carcinoma

## MATERIAL AND METHODS

The study included a group of 50 healthy normal individuals aged from 2? to 88 years and a group of ?8 patients aged from 29 to 75 years with cervical uterine carcinomas All the cancer patients had a proven histological diagnosis and were staged according to the TNM classification (12) No premalignant changes of the cervix such as squamous cell metaplasia or dysplasia was included in the study Approximately 40% of the patients (12 out of ?8) exhibited minimal malignant tumor development being classified as stage I B Of the remaining patients 2 were classified as stage II A 2

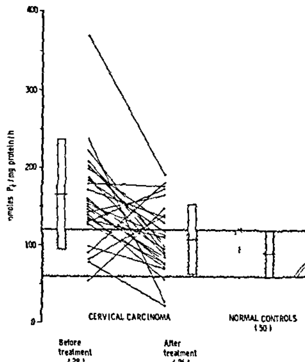



*Fig 1* Oligomycin sensitive ATP ase activities of lymphocytes from patients with cervical carcinoma before and after treatment and of lymphocytes from normal healthy controls The columns are the means ±1 S D The normal range is shaded The number of patients and of controls investigated is given in brackets

as Stage II B 1 as stage III A 7 as stage III B and finally 4 as stage IV The lymphocyte ATP ase activity was determined shortly before and 6–12 weeks after treatment This was in all cases a combination of radium treatment and external irradiation

The technique of lymphocyte isolation from peripheral blood has been changed from the one previously described in order to obtain a further purification of the lymphocytes from platelets and red cells A 50 ml sample of venous blood is drawn with a plastic syringe and immediately heparinized (8 I U per ml) within the syringe The blood sample is then passed slowly through a 5 cm long column of washed 0 1 mm glass beads in a 10 ml plastic syringe in order to remove the most sticky population of platelets This prevents aggregation of platelets during the subsequent isopycnic centrifugation which could cause alterations in their sedimentation characteristics The blood is then diluted with 3 volumes 0 87% NaCl also heparinized with 8 I U per ml and the total volume is divided into 40 ml fractions in 50 ml centrifuge tubes Ten ml of a 9% Ficoll/33 9% diatrizoate sodium mixture (v/v 24 10) is carefully injected under the diluted blood and the tubes spun at 650×g for 20 min The lymphocyte interphase is then harvested with a Pasteur pipette and after an intermediate wash in 0 87% NaCl the lymphocytes which are still contaminated with some platelets and red cells are suspended in 5 ml 10% sucrose and layered upon a discontinuous sucrose gradient of 25 ml 16% sucrose and 4 ml 20% sucrose The tube is then spun for 15 min at 150×g and the lymphocytes can now be obtained from the bottom as a pure cell suspension free from platelets and only in very rare cases contaminated with some red cells In such cases a 5 min exposure to a 1% ammonium oxalate solution will lyse the remaining red cells completely The lymphocytes are now counted suspended as a concentration of about $10^{8}$ lymphocytes per ml in 0 25 M sucrose and sonicated for 2×15 sec with a MSE sonicator Great care is taken to keep the tubes and the cell suspension cooled before and during the sonication The lymphocyte homogenate is then spun at 1000×g for 10 min in a refrigerated centrifuge and the supernatant used for the ATP ase assay The protein concentration of the supernatant is determined by the method of Lowry et al (13) The ATP ase assay itself is unchanged from previous publications (3 5 6) The enzymatic liberation of inorganic phosphate ($P_i$) from the substrate Tris-ATP is determined and the results are corrected for the lymphocyte endogenous content of $P_i$ and the nonenzymatic $P_i$-release during the assay (1 h) Double incubations and double determinations are used throughout the whole procedure The results are expressed as nmoles $P_i$ liberated per hour per mg protein of the homogenate

In characterizing the lymphocyte population T lymphocytes were identified by their ability to form rosettes with sheep red cells (7) in Hanks solution after incubation with the red cells for 15 min at 4 C followed by 10 min in a shaker at room temperature The number of lymphocytes having attached 4 or more red cells to their membrane were counted as positive The B-cells were identified by their ability to bind fluorescent antibodies (antihuman IgG IgM & IgA) to their membrane (8) By fluorescence microscopy a total of 200 lymphocytes were counted and the percentage of cells showing a clearly visible membrane fluorescence with either one of the anti human antibodies were recorded as B lymphocytes

All reagents used were of analytical grade Ficoll was purchased from Pharmacia Sweden and Tris ATP from Sigma Chemical Company St Louis Mo USA The fluorescent anti human antibodies (MF 03 04 05) were from Wellcome Research Laboratories Beckenham England

## RESULTS

A scatter diagram of the oligomycin sensitive mitochondria related ATP ase activity of lymphocytes from 28 patients with cervical carcinomas of the uterus and from 50 normals is presented in Fig 1 The lymphocyte ATP ase activity before treatment was 166 3±70 6 (mean ±1 S D ) nmoles $P_i$ per mg protein per hour which is significantly higher ($0.01<p<0.02$) than the activity in lymphocytes from normal controls (91 3±33 3) After treatment the activity in most cases decreased to a lower level with a group mean activity of 108 0±45 8 nmoles $P_i$ per mg protein per hour Before treatment 5 patients had lymphocyte ATP ase activities within the nor

mal range compared to 17 patients after the treatment

In all patients but two the effect of the treatment given was recorded as complete with no signs of local relapse or of developing metastases The two patients whose cervical carcinoma was not successfully treated as evaluated 6–12 weeks after treatment had unchanged high or rising ATPase activity at the follow up examination In 4 other patients the activity rose after treatment in three of these from within the normal range before treatment to clearly elevated values after treatment. These four patients were considered to be clinically cured

Neither in the normal group nor in the group of patients a correlation between age and the lymphocyte ATPase activity was demonstrated Similarly there was no correlation between the stages of malignancy and the corresponding lymphocyte ATPase activities

The additional experiments carried out in this study in order to further characterize the cell suspensions showed that an average of 51% of the lymphocytes in the final cell suspensions prepared by the present technique could be characterized as T-cells by rosette formation with sheep red cells (7) and 30% of the lymphocytes was characterized as surface immunoglobulin carrying B-cells (8) The remaining 19% of the lymphocytes could not be identified by these means No significant difference between the relative proportion of B & T cells in the patient group and the control group was found

## DISCUSSION

It should be noted that the pre treatment ATPase activities are very scattered as compared with the post treatment values and the normal controls (Fig 1) In a total of 12 patients with carcinoma (43%) the values are overlapping the highest non malignant control values recorded but 82% of the pre treatment values of the cancer group are higher than the normal mean ±1 S D

The decline in the ATPase activity of the peripheral lymphocytes from cancer patients after treatment is obvious In approximately 80% of the ATPase activity dropped significantly In one half of the patients the activity dropped from pre treatment elevated levels to activities within the normal range In two of the 6 patients who had unchanged or higher lymphocyte ATPase activity 6–12 weeks after treatment routine physical examination showed no effect of the radiation therapy In the remaining 4 patients with rising ATPase activities however the effect of the treatment was recorded as complete with no signs of relapse or developing metastases These 4 patients will be followed carefully to see if symptoms of relapse or metastases should develop

The normal range of the lymphocyte ATPase activity differs from that previously published probably because of several technical changes in the method primarily the much higher purification of the lymphocytes This could have led to a bigger percentage of the lymphocytes being non functioning or killed but the previously higher ATPase activity level recorded could also be due to some of the activity originating from non lymphoid cells primarily platelets present in the final cell preparations The homogenization of the cells by sonication instead of using a rotating glass pestel homogenizor also may account for a part of the difference between the present results and the results previously obtained

Since a substantial number of patients with cervical uterine carcinomas (23 out of 28) exhibited elevated lymphocyte ATPase activity (mean ±1 S D ) the important question of diagnostic significance could be raised The overlapping with the highest normal control values makes it impossible to claim that every above normal value should be diagnostic for cervical cancer A lymphocyte ATPase activity of more than 50% above the normal mean however which accounts for almost 70% of the ATPase activities in the present pre treatment group seems to be diagnostic for cancer Extremely high activities certainly would be diagnostic as false positive values are very few are only moderately elevated and hitherto have been found only in patients with hepatic amoebiasis and ulcerative colitis A normal lymphocyte ATPase activity does not exclude the possibility of malignancy in a patient with a cervical erosion or tumor but a repeated determination would bring the chance for a false negative result down to around 7% (2 out of 28)

Among other biochemical screening tests for cancer the most important seems to be a demonstration of elevated serum concentrations of the carcino-embryonal antigen (CEA) which already for some time has been in clinical use A negative CEA test does not exclude cancer and only very much elevated values are diagnostic (14) In contrast to the lymphocyte ATPase activity however the CEA test is almost invalidated by a high rate of false

positive scores among a number of non malignant diseases and its primary use is confined to entoder mally derived tissues

The lymphocyte ATP ase activity has previously been found significantly elevated in lung carcinoma gastrointestinal carcinomas and breast carcinomas (6) The test also appears to be of diagnostic and perhaps prognostic significance in cancer of the uterine cervix

## ACKNOWLEDGEMENTS

This study was supported by grants from Statens Lægevidenskabelige Forskningsråd and Landsforeningen til Kræftens Bekæmpelse (The Danish National Cancer Fund) We are indebted to miss Kate Sommer for expert technical assistance and we also want to thank our col leagues at the Radium Station Aarhus for kind cooperation

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*Submitted for publication Febr 2 1974*

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Acta Obstet Gynec Scand 54 227–230 1975

# SITE OF OVULATION AND ECTOPIC PREGNANCY

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*Abstract* In a study of 130 tubal pregnancies the relationships as to location of the corpus luteum the implantation site and the gross findings of the non pregnant fallopian tube were analyzed A contralateral corpus luteum was found in 20% of the cases Hydrosalpinx peritubal adhesions and/or thickening of the tubal wall were observed in 33% of the non pregnant tubes Grouping by implantation site—ipsilateral or contralateral corpus luteum—showed no statistical differences The incidence of chronic pelvic inflammatory diseases was no greater in the subjects above 30 years of age and those having had more than [illegible] pregnancies The results suggest that tube locking of the ovum sometimes a result of previous tubal inflammatory disease sometimes a result of supposed insufficiency of tubal peristalsis was the major cause of tubal gestation External migration of the ovum alone may not be an important factor in the genesis of tubal pregnancy

It is assumed that the fimbriae of the fallopian tube partly envelop the ovary at the time of ovulation The ovum is discharged near or into the opening of the fallopian tube and the beating of the tubal cilia carry the ovum into the osteum of the tubal lumen and into the ampulla Generally speaking ovulation fertilization and transportation of the ovum appear to occur in the ovary and uterine tube on the same side

In some tubal pregnancies the corpus luteum of pregnancy has been recognized on the side opposite to the ectopic pregnancy Berlind (1) reported that the corpus luteum was found on the contralateral side in 24 of 50 ectopic pregnancies and suggested that transmigration of the fertilized ovum may be an important factor in the production of tubal gestation The possibility of transmigration of ova in normal pregnancies is not certain and at most it is considered to be a relatively rare event The present study was carried out to evaluate the probable cause of the corpus luteum being contralateral in tubal pregnancies and to review the possibility of transmigration of the ova occurring in normal pregnancy

## MATERIALS AND RESULTS

The materials utilized in this study consist of 130 women operated on for tubal pregnancy at the Tokyo Medical and Dental University Hospital The data were statistically analyzed by the chi square test

One hundred and one of the 130 patients (77.7%) had a history of previous pregnancies Ages ranged from [illegible] to 44 years with the largest number (42 or 32.3%) occurring in the 25 to 29 year age group To assist in reference to the location of the tubal pregnancy the fallopian tube may be divided into proximal middle and distal thirds In 63 cases the pregnancy was in the distal third of the fallopian tube (48.5%) in 45 the middle third (34.6%) and in 22 the proximal third (16.9%) Among the proximal third cases are included five pregnancies classified as interstitial in location

The site of the corpus luteum and gross findings of the fallopian tubes were checked during the operation The corpus luteum was on the right side in 57 patients on the left side in 61 cases in 12 cases the side was indeterminable The pregnancy was in the right tube in 68 cases (52.3%) and in the left tube in 62 cases (47.7%) The corpus luteum was contralateral in 26 cases (20.0%) ipsilateral in 92 cases (70.8%) and in 12 cases the corpus luteum side was indeterminable In 15 cases external migration of an ovum from the left ovary to the right tube had taken place and in 11 from the right ovary to the left tube (Table I)

Table I *Site of the pregnant tube and corpus luteum*

Tubal pregnancy was in the left tube in 62 cases and in the right tube in 68 cases In 15 cases external migration of an ovum from the left ovary to the right tube had taken place and in 11 cases from the right ovary to the left tube

| Site of pregnant tube | Site of corpus luteum | | | |
|---|---|---|---|---|
| | Left | Right | Indeterminable | Total |
| Left | 46 | 11 | 5 | 62 (47.7%) |
| Right | 15 | 46 | 7 | 68 (52.3%) |
| Total | 61 (46.9%) | 57 (43.8%) | 12 | 130 (100%) |

In 24 of 130 patients (18.5%) hydrosalpinx and complete closure of the fimbriae were noted in 19 (14.6%) peritubal adhesions or marked thickening of the tubal wall was observed and in 11 (8.5%) a tube had been resected at an earlier operation In the other 76 patients (58.5%) the contralateral tubes appeared to be normal on inspection (Table II) Nineteen patients with peritubal adhesions noted at the time of laparotomy later had two ectopic pregnancies and one normal intra-uterine pregnancy Incompletely performed follow-up studies disclosed that 76 patients who had had normal appearing contralateral tubes later had two ectopic pregnancies and 13 normal pregnancies

The distribution of the implantation site in the ipsilateral and contralateral corpus luteum groups was not statistically different at the 1% level of significance (Table III) However the incidence of implantation in the mid portion of the tube was less and in the distal third greater in the group over 30 years of age than in the group 20 to 29 years of age (Table IV) The site of nidation did not seem to be affected by the number of previous pregnancies

The incidence of hydrosalpinx complete closure of the fimbriae peritubal adhesions and marked thickening of the tubal wall was no greater in the patients above 30 years of age and those who had had more than two pregnancies than in the group less than 29 years of age and those who had had fewer than 3 pregnancies

## DISCUSSION

The 20% incidence of contralateral corpora lutea in tubal gestation was less than that reported by Berlind (1) and Breen (3) and more than Kleiner (8) In our contralateral corpus luteum group 5 patients were found to have complete closure of the fimbriae and one to have had a previous tubal resection on the side of ovulation (Table II) Thus there was no chance for the ovum in these 6 patients to pass through an ipsilateral uterine tube The adjusted incidence of unexplainable transmigration of the fertilized ovum is therefore 17.9% in our group of patients

The same incidence of corpora lutea on the right and left sides (43.8% and 46.9% respectively) suggests that ovulation generally occurs alternatively first on one side and then on the other It is also to be assumed that the basic cause of tubal pregnancy is any condition which prevents or retards the passage of the fertilized ovum into the uterine cavity The most common morphologic finding is reported to be chronic endosalpingitis (3 8) Hydrosalpinx closure of the fimbriae and/or moderate or marked adhesions of the non pregnant tubes were seen in 33% of our cases There was

Table II *Site of the corpus luteum and gross findings in the non pregnant tube*

Hydrosalpinx closure of the fimbriae and/or adhesions of the non pregnant tubes were seen in 33%

| Site of corpus luteum | Gross findings in non pregnant tube | | | | |
|---|---|---|---|---|---|
| | Normal | Hydro salpinx | Adhesions | Resection | Total |
| Ipsilateral | 52 | 18 | 13 | 9 | 92 |
| Contralateral | 17 | 5 | 3 | 1 | 26 |
| Indeterminable | 7 | 1 | 3 | 1 | 12 |
| Total | 76 (58.5) | 4 (18.5%) | 19 (14.6) | 11 (8.5%) | 130 (100%) |

Table III *Site of the corpus luteum and implantation*

Distribution of the implantation site in ipsilateral and contralateral corpus luteum groups showed no statistical differences

| *Site of* corpus luteum | Site of implantation | | | Total |
|---|---|---|---|---|
| | Distal | Middle | Proximal | |
| Ipsilateral | 46 | 32 | 14 | 9? (70.8%) |
| Contralateral | 11 | 9 | 6 | ?6 (?0.0%) |
| Indeterminable | 6 | 4 | 2 | 1? |
| Total | 63 (48.5%) | 45 (34.6%) | 2? (16.9%) | 130 (100%) |

*no tendency toward an increase in chronic tubal inflammatory disease with advancing age in the multigravida. An increased incidence of implantation in the distal and proximal parts of the tube* were exhibited by patients more than 30 years of age (Table IV). The possibility still remains, however, that the normal appearance of the non-pregnant tube does not reveal the true condition of the endosalpinx. No congenital tubal abnormalities were observed in the material studied.

Table V shows the number of pregnancies with simple and external migration of the ovum. Calculations were based on the following facts and assumptions: (*a*) in 69 of our cases the non-pregnant tubes were found to be normal on inspection, (*b*) the incidence of external migration in our series was 17.9%, (*c*) the incidence of ectopic pregnancy is 1.15% of deliveries (3), (*d*) the incidence of spontaneous abortion is 12% (4). The number of pregnancies in which 69 ectopic pregnancies occurred was calculated as follows: 69×0.88/0.0115, and total number of contralateral

Table IV *Age and implantation site*

Incidence of implantation in the middle third of the tube was less and in the distal third greater in the group over 30 years of age than in the group 20 to 29 years of age

| Age | Site of implantation | | | Total |
|---|---|---|---|---|
| | Distal | Middle | Proximal | |
| 20–29 | 20 (37.7%) | 26 (49.1%) | 7 ([illegible]) | 53 (100%) |
| 30–44 | 43 (55.8%) | 19 (24.7%) | 15 (19.5%) | 77 (100%) |
| Total | 63 | 45 | 2? | 130 |

Table V *Supposed number of pregnancies with ipsilateral and contralateral corpora lutea in ectopic and normal pregnancies calculated from our 69 cases ectopic pregnancies*

Incidence of ectopic pregnancy in ipsilateral and contralateral corpus luteum groups showed no statistical differences

| Site of corpus luteum | Site of pregnancy | | Total |
|---|---|---|---|
| | Ectopic | Normal | |
| Ipsilateral | 5? (0.93%) | 5 549 | 5 601 (100%) |
| Contralateral | 17 (1.39%) | 1 ?00 | 1 ?17 (100%) |
| Total | 69 | 6 749 | 6 818 |

*corpora lutea was 6818×0.179 equaling 1217.* No significant difference in the incidence of tubal pregnancy was demonstrated between the suspected ipsilateral and contralateral corpus luteum groups.

Figure 1 shows a basal body temperature chart (BBT) of one patient out of 6 cases having BBT chart and who later had a tubal pregnancy. The patient was 24 years old and had had 4 abortions previously. Her menstrual cycle usually lasted 32 to 35 days. *In the menstrual cycle under consideration* ovulation occurred on day 17 and vaginal spotting and slight abdominal pain began from day 33. *She was admitted to the hospital* and on day 43 a urine pregnancy test was positive. She was operated upon on day 48 and the corpus luteum was observed on the ipsilateral side. The result seems to offer no support for the postulation by Iffy (6) that delayed ovulation is the main cause of tubal gestation.

We propose that (i) partial obstruction of the tubal lumen, (ii) peristaltic insufficiency of the tubal muscle, or (iii) further development of the ovum before passing through the uterine tube may *be involved in the cause of tubal gestations. Other* reports (2, 3) as well as this study have shown that the implantation site of tubal pregnancy was most commonly in the distal third, less frequently in the proximal third of the uterine tubes. Furthermore, it is evident that inflammatory diseases of the uterine tube were not found in all the cases. The same incidence of implantation site in both the contralateral and ipsilateral corpus luteum groups suggests that the fallopian tube on either the same side as the ovulation or the opposite side probably had the same pathological condition in transporting

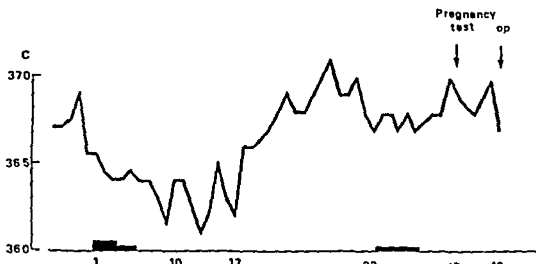



Fig. 1 Basal body temperature chart of a patient with left tubal pregnancy 24-year-old gravida 4 para 0 abortion 4 female Her menstrual cycle lasted 3? to 35 days Ovulation occurred on day 17 Vaginal spotting and abdominal pain began from day 33 A urine pregnancy test was positive on day 43 The patient was operated upon on day 48

of the ovum Therefore successful normal pregnancy after tubal gestation may be limited to some extent

Many investigators have considered that it is the trophoblast which implants itself The fertilized human ovum usually enters the uterine cavity as a morula 3 or 4 days after ovulation For 6 or 7 days after ovulation the trophoblast can attach to the surface of the endometrial epithelium and the ... yst is ready to implant (5) The non specific ... e of nidation is evidenced by the fact that ... blastocyst can readily imbed itself in a variety of extra uterine sites

Transportation of the ovum is largely dependent on the peristaltic activity of the uterine tube Insufficient peristalsis of the tube retards the passage of the ovum and might be expected to increase the incidence of implantation at the proximal or mid portion of the tube but this did not appear to occur in our cases Insufficient movement of the tubal cilia and consequent delay in the transportation of the ovum into the tubal osteum is supposed to be a reason for pregnancy in the distal and mid portions of the tube

The results show that the most common cause of tubal pregnancy is tube locking (7) of ova in the ampulla sometimes the result of obstruction of the lumen of the fallopian tube and sometimes by supposed tubal peristaltic insufficiency It is possible that external migration of the ova plays no major role in the pathogenesis of tubal pregnancy

## ACKNOWLEDGEMENT

Partly supported by grants in aid for Developmental Scientific Research from the Education Ministry Japan The authors are indebted to Drs W Locke and J Nerness for reviewing the manuscript

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*Submitted for publication Febr 4 1974*

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Acta Obstet Gynec Scand 54 231-235 1975

# AN IMPROVED METHOD OF EPIDURAL ANALGESIA WITH REDUCED INSTRUMENTAL DELIVERY RATE

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*Abstract* A detailed study on a modified technique of epidural analgesia (EDA) for pain relief in obstetrics has been performed The aim of the modifications was to reduce the number of instrumental deliveries and at the same time to make the delivery as smooth as possible for the baby This was achieved by the use of an anaesthetic with a favourable ratio between neonatal and maternal plasma levels (Bupivacaine) in low concentration (0 25%) A special technique of injection enabled us to limit the extent of the blockade An epidural catheter was inserted between L2 and L3 and moved upwards 20 cm into the epidural space 8–10 ml of the solution was then injected after a test dose The blockade was continued by the repeated injection of smaller doses As judged by the skin anaesthetic zones and by obstetric examinations the patient was gradually positioned during labour from supine to half sitting The catheter was withdrawn at the end of the first stage of labour so that the lower sacral segments could be blocked A group of 100 patients treated with the technique described was followed The number of instrumental deliveries in the present series (15%) was significantly lower than in similar series reported in the literature The group of 100 patients treated with modified EDA was also compared with 100 control patients who received only conventional treatment without EDA There were no differences in the number of abnormal presentations while the number of caesarean sections and cases of atonic post partum bleeding was insignificantly lower in the EDA group The difference in the number of instrumental deliveries—although somewhat higher in the EDA group—was not statistically significant The total length of labour was prolonged in the EDA group but EDA was not necessarily responsible for this undesired effect The clinical status of the babies was found to be better after EDA than in the control group as judged from Apgar score Furthermore fetal bradycardia was significantly reduced with EDA It is concluded that EDA as used in this study not only is a preferable way of achieving pain relief in the mother but also offers a means of facilitating the birth process for the infant reducing the incidence of pre and post natal asphyxia

Among different approaches to pain relief in obstetrics epidural analgesia (EDA) is considered to be the most effective method (2 3 4 5 7) Some side-effects of EDA however have been factors in preventing a wider application of the method The criticism that there are an increased number of instrumental deliveries has often been noted in connection with EDA (see Table IV)

The increased instrumental delivery rate is commonly considered as a potential risk for the baby Therefore the present study was aimed to reduce the instrumental delivery rate by an improved method of EDA The technique used consists in the use of a low concentration of local anaesthetic the site of injection of which was varied with the progress of labour This accurate siting of the anaesthetic solution was obtained by the gradual change in position of the patient To test the effect of this variation in technique a detailed study was performed The course of deliveries as well as clinical findings on babies in a group treated with EDA and in a group of deliveries with conventional pain relief were compared The technique of EDA and the results as obtained with the improved technique will be described

## MATERIAL AND METHODS

### *Selection of cases*

There were two groups of patients in this study The first group consisted of 100 women who delivered with EDA The selection of patients for EDA was done according to following indications (1) unusually painful labour (?) explicit wish of the patient (3) induction of labour (particularly with Rh immunisation or postmaturity) (4) drug addiction and/or other psychiatric diseases (5) rigid cervix or cervix after conisation (6) incoordinate uterine action and (7) breech presentation

As a control group 100 women who delivered during the same time period were used They were selected at random from the diary at the delivery suite The control group

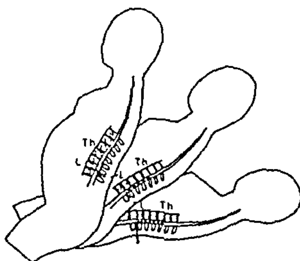



*Fig 1* Technique of injection The catheter is introduced by means of a needle inserted between L2 and L3 and moved upwards 20 cm into the epidural space The patient is gradually positioned from supine to half sitting At the end of the first stage of labour the catheter is withdrawn 8–10 cm so that the lower segments of the cord can be blocked

did not differ from the total material of the clinic with regard to the frequency of vacuum extractions or caesarean sections as compared by the $\chi^2$ test Conventional methods for pain relief were administered in this group ($N_2O$ + $O_2$ Pethidine Pentazocine Diazepam Methoxyfluorane pudendal block infiltration of perineum) Gestational age at the time of labour ranged between 31 and 41 weeks Data the groups are shown in Table I Before any analgesia started oxytocin was administered in 23 patients from group and in 46 patients from the EDA group

*d of injection*

Bupivacaine solution (0 25 without epinephrine) was injected through an epidural catheter which was introduced by means of a needle (Tuohy Flower type) inserted between L2–L3 The catheter was moved upwards 20 cm into the epidural space and 8–10 ml of the solution injected after a testdose (2–3 ml 0 25% Bupivacaine) The blockade was continued by the repeated injection of smaller doses (approximately 5–6 ml each but a total of not more than 200 ml within 12 hours) As judged by the skin anaesthetic

Table I *Basic data on EDA and control groups*

| | Total no | No of primi gravidas | Age years Mean | (S D) |
|---|---|---|---|---|
| Controls | 100 | 47 | 27 | (4 7) |
| EDA | 100 | 75 | 24 | (4 ?) |

zones and by obstetric examinations the patient was gradually positioned from supine to half sitting (as the cervix opened so more of a sitting position was used) The catheter was withdrawn 8–10 cm at the end of the first stage of labour so that the lower sacral segments could be blocked

*Statistical evaluation of results*

For the purpose of statistical comparison correlation coefficients were used The results were then checked by $\chi^2$ test or by $t$ test according to the qualitative or quantitative nature of the data examined

## RESULTS

*Effects of EDA on the course of delivery*

*(a) Instrumental deliveries Caesarean sections and length of the delivery comparison between EDA group and controls* The number of instrumental deliveries (i e vacuum extractions) the number of Caesarean sections and the total length of the first and second stages of labour are shown in Table II

The number of instrumental deliveries was higher in the EDA group as compared with controls but the difference was not statistically significant The number of Caeserean sections was lower than in controls Even this difference was not statistically significant The total length of the first and second stage of labour was significantly prolonged in the EDA group In the control group there were 4 cases of atonic post portum bleeding after delivery but none in the EDA group

*(b) Occipito-posterior presentation in deliveries with EDA* No significant difference was noted be

Table II *Instrumental deliveries Caesarean sections and the duration of labour*

| | EDA group | Controls | Significance of the difference |
|---|---|---|---|
| Total no | 100 | 100 | – |
| No of instrumental deliveries | 15 | 7 | $\chi^2$=3 27 (non significant) |
| No of Caesarean sections | 4 | 8 | $\chi^2$=1 4? (non significant) |
| Length of the delivery (mean value in hours) | 13 30 (S D 5 66) | 9 ?9 (S D 4 65) | $P$<0 01 ($t$ value 5 474) |

tween EDA group (6 cases) and controls (7 cases) in the rate of abnormal presentation

*Effects of EDA on the babies*

Apgar score fetal bradycardia and the occurrence of discoloured amniotic fluid in both groups were noted The results as obtained in the EDA group and in controls are compared in Table III As shown all three indicators were more favourable in the EDA group The fourth indicator (discoloured amniotic fluid) was somewhat more favourable in the EDA group but the difference was not statistically significant

## DISCUSSION

There are several factors which prevent a wider use of EDA for pain relief during delivery Although the economical and organisational problems are of great importance subjective factors and distrust in both patients and the staff may be decisive in the matter Sometimes the negative attitude is without any rationale ( the delivery is a physiological event any artificial intervention is unnecessary the pain during delivery supports later emotional contacts between the mother and the child ) However most often any type of analgesia was regarded as a potential risk factor either for the patient or for the fetus The increased instrumental delivery rate was considered to be an important risk factor with EDA (9 13 17 24) In fact the relationship between an increased instrumental delivery rate and some unfavourable influence on the baby is very doubtful as a result of EDA (see Table III) Anyway the study presented showed that the instrumental delivery rate might be lowered significantly as compared with

*Table III Apgar score decrease of the fetal heart rate and occurrence of meconium stained amniotic fluid*

| | EDA group | Controls | |
|---|---|---|---|
| Total no | 100 | 100 | |
| Apgar score ≤ 8 at one minute | 4 | 17 | $\chi^2=8.99$ ($P<0.01$) |
| Apgar score ≤ 8 at five minutes | 0 | 4 | $\chi^2=4.08$ ($P<0.05$) |
| Fetal bradycardia ≤110 | 8 | 19 | $\chi^2=5.18$ ($P<0.05$) |
| Meconium-stained amniotic fluid | 10 | 14 | $\chi^2=0.76$ (non significant) |

*Table IV Instrumental delivery rate as described in the literature*

| Author | | No of cases | No of instrumental deliveries (%) |
|---|---|---|---|
| Duthie Wyman (9) | 1968 | 30 | 93 |
| D Vere et al (23) | 1968 | 214 | 91 |
| Browner Catton (5) | 1971 | 170 | 84 1 |
| Epstein et al (11) | 1968 | 10 000 | 75 |
| Doughty (8) | 1965 | 172 | 75 |
| Hehre et al (13) | 1969 | 61 | 57 |
| Crawford (6) | 1972 | 923 | 50 1 |
| Belfrage Raabe (2) | 1970 | 114 | 39 5 |
| Ekholm et al (10) | 1970 | 100 | 36 0 |
| Kandela Malmnäs (14) | 1972 | 120 | 30 5 |
| Noble et al (19) | 1971 | 100 | 30 |
| Mueller (18) | 1971 | 100 | 28 |
| Doughty (8) | 1969 | 425 | 24 |
| Maltau (16) | 1972 | 150 | 14 |
| Own results | 1973 | 100 | 15 |

that reported in the literature (Table IV) With one exception Maltau (16) the difference between the results of other authors and our own results was highly significant ($P<0.01$)

Moreover a comparison between instrumental delivery rates in the EDA group and in a control group was made Although the number of instrumental deliveries was higher in the EDA group the difference did not reach a statistically significant level (Table II) There were some substantial differences in composition of the two groups While the control group was selected to be a representative sample of the deliveries of the clinic the EDA-group consisted of patients with higher risk for complications There are both medical and ethical reasons why a pure random selection of patients for EDA could not be done There were clear obstetrical indications or very painful labour and in some cases it was an explicit wish of the patient to get an EDA Accordingly the number of primigravidas was higher in the EDA group (75% in EDA-group versus 47% in control-group) All those factors certainly influenced the instrumental delivery rate If the number of primi and multigravidas was made the same in both groups (i e 144 patients instead of 200 with 47 primi and 25 multigravidas in each group) the differences became less obvious for instance there were 12 instrumental deliveries in the EDA group and 7 in controls ($\chi=1.51$ as compared with $\chi^2=3.27$ in unselected material) Further diminishing of

the differences between EDA-group and controls might be expected if only randomly selected patients would be compared

There were two important points in the technique of EDA which were probably responsible for the favourable results Firstly we aimed to limit the blockade The level of the blockade was gradually changed during the delivery Therefore the catheter was placed as high as Th 12 – Th 11 and withdrawn 10 cm in the later stages of the delivery The extent and level of the blockade was also controlled by positioning the patient The position was successively changed after each injection from supine position to half sitting The effect was checked by testing the skin anaesthetic zones (which should be limited to a bikini type of anaesthesia in the second stage) It was found that the less extensive but correctly placed blockade was sufficient for pain relief Moreover the motor activity of pelvic muscles was preserved during the first stage of delivery In this way some of the abnormal influences on the physiological mechanism during delivery could be prevented In earlier techniques of EDA an increased rate of abnormal presentation during delivery was noticed (2 5) probably due to blockade of pelvic floor muscles In the present series there were 6 cases of abnormal presentation in the FDA group and 7 cases in controls Thus EDA showed us unfavourable influence

Secondly attention was paid to the concentration type of the anaesthetic solution A review of the ature shows that the best results appear to be achieved with 0 25 % Bupivacaine The choice of the proper solution is based mainly on practical experience the theoretical background being under discussion According to one hypothesis the motor sensory and autonomic nerve fibres differ in their sensitivity to anaesthetics according to their anatomical structure Thus a more selective blockade of sensitive impulses may be reached with lower concentrations of the drug

The statistical evaluation confirmed that labour was significantly prolonged in the EDA group as compared with controls This result does not mean necessarily that the use of EDA was the cause of the slow progress of labour As already mentioned the EDA group was not exactly equivalent to the control group since there were many patients with primary obstetric pathology in the first group Moreover the number of primigravidas was higher in the EDA group On the other hand some residual undesired effect of EDA could not be excluded even with the improved method of injection Also some prolongation of labour is not necessarily undesirable

There is no doubt that some patients would prefer EDA even with a longer labour Since the treatment was advantageous for the mothers the influence on babies should also be discussed All three factors studied had something to do with the babies well fare during the delivery (Apgar score occurrence of fetal bradycardia and meconium stained liqour during the delivery) and the results favoured the EDA group As shown in Table III the difference was statistically significant for two of the three factors (Apgar score and fetal bradycardia) Apgar score has been shown to correlate with neonatal morbidity and future intellectual development (1 19 21) Indeed the fetus may fare better with EDA than with conventional analgesic methods

An elimination of maternal hyperventilation together with a less precipitate second stage of labour *might explain this positive effect of EDA (8 1? 15)* Besides the concentration the choice of anaesthetic seems to be of some importance too The authors who described bad effects of EDA on babies well fare (13 24) used prilocaine lidocaine mepivacaine in all cases in relatively high concentrations The good effects of EDA with bupivacaine as found in this study are supported by statements of other authors (2 8 16 19 21)

In all cases reported in these papers Bupivacaine was used The effect may be explained by the study reported by Reynolds (22) In her series the mean ratios of neonatal to maternal plasma concentration were significantly lower for Bupivacaine as compared with similar ratios for Lignocaine and Mepivacaine Moreover Bupivacaine does not tend to accumulate in the fetus Thus the benefit given by other factors cannot be disturbed by some toxic effects of the anaesthetics

In summary EDA should be considered not only as an effective pain relief and a useful therapeutic procedure in some cases but also as a technique aimed at preserving the baby s well fare as well

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*Submitted for publication Febr 12 1974*

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Acta Obstet Gynec Scand 54 237–239 1975

# DIAZEPAM AS A SEDATIVE IN INDUCED ABORTION

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*Abstract* In 136 patients who underwent induced abortion by the vacuum curettage method under local anaesthesia (paracervical block) the effect of 10 mg diazepam intravenously as preoperative sedative was investigated for its ability to abolish the subjective experience of pain The trial was carried out as a paired sample random allocation double blind fixed dose trial and the statistical method was sequential analysis Thirty six pairs showed no difference and were excluded After 32 pairs (24 A preferences and 8 O preferences) diazepam showed a significant superiority to placebo ($p<0.05$) (Fig 1) Amnesia for the procedure was reported by about one-quarter of the diazepam-treated patients and in practically all cases the sedative effect had subsided 1–3 hours after the operation

Diazepam has in recent years gained increasing ground prior to minor surgery and disagreeable diagnostic procedures (5 6 7) It has been emphasized in particular that it reduces pain and discomfort and that in many cases it produces total amnesia for what has been performed (7 8)

During induced abortion carried out as vaccum curettage under local anaesthesia (paracervical block) many patients show very marked reactions which render the procedure difficult and—more important still—leave the patient with a disagreeable experience of pain

It therefore seemed reasonable to investigate whether diazepam by the intravenous route was able to abolish the pain caused by this procedure

## MATERIAL AND METHOD

The trial was performed at the Department of Gynaecology and Obstetrics in Øresundshospitalet Copenhagen during the period December 1970 to December 1971 as a paired sample random allocation double blind fixed dose trial (1 9)

The patients were admitted to the trial at random among those in whom the stage of gestation permitted vacuum curettage i e not more advanced than the 12th week A total of 136 patients were included

The results were calculated statistically by the sequential analysis (1 9) based on pairs of patients given different treatment (in the present trial diazepam/placebo) Report of pain only by the diazepam-treated patient was designated O preference whereas report of pain only by the placebo-treated patient was designated A preference The patients were classified according to whether they answered yes or no to the direct question put 1–3 hours postoperatively Was the operation painful?

The patients were coded and paired one half receiving diazepam and the other half placebo The code numbers were entered on forms which were then mixed at random for 5 pairs together

These forms consisted of two parts Part 1 gave general data about the patient (name age weight parity previous induced abortion and daily use of tranquillizers) as well as the surgeon s impression relating to the effect of the drug given Part 2 was used for recording the patient s reaction to the procedure 1–3 hours later The persons who completed part 2 did not see part 1 Data for part 2 were collected by nurses who asked short non leading questions such as Did it hurt? Do you remember what happened? In this way the data were less biased than they would have been if the question had been asked by the surgeon At the same time the patient s level of consciousness was recorded i e whether she was awake replied adequately and was able to get out of bed unaided

In all cases the surgical procedure was the same viz curettage according to principles described previously (3) About half an hour before the procedure the patient received premedication with 50 mg pethidine subcutaneously On the couch 2 ml (10 mg) diazepam (Stesolid®) or a corresponding dose of placebo (the solvent without diazepam) was administered intravenously Three minutes later a paracervical block was established by quadrant injections each of 5 ml 1% mepivacaine hydrochloride (Carbocaine®) After another three minutes the cervical canal was dilated by a Hegar dilatator usually to one number larger than the suction curette Thereafter the uterine contents were removed by the suction curette and at the same time 0 2 mg methyl ergometrine maleate (Methergin®) was given intravenously

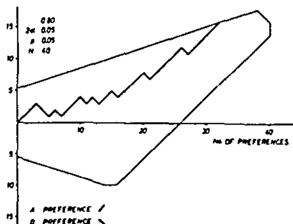



*Fig 1* Closed sequential diagram for one sided test After 32 preferences the figure shows that diazepam is significantly superior to placebo

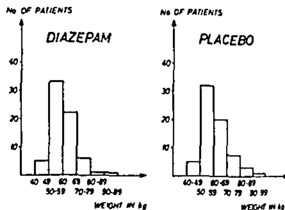



*Fig 2* Distribution of patients by body weight in the two groups diazepam/placebo

## RESULTS

A total of 136 patients were admitted to the trial 36 pairs showed no difference and were therefore excluded from the analysis Fig 1 presents the result showing that the curve crosses the upper limit of the sequential diagram after 32 preferences (24 A preferences and 8 O preferences) Thus diazepam abolished the pain experience of induced abortion as described above and the superiority of diazepam is statistically significant $p$ value below 0 05

Table 1 sets out the comparability of the two groups (treated with diazepam and with placebo) It shows practically the same distribution by age parity previous induced abortion and daily intake of tranquillizers By body weight the patients showed an entirely identical distribution (cf Fig 2)

Eighteen of the 68 patients treated with diazepam reported total amnesia for the procedure

Table 1 *Distribution of patients in the two groups diazepam/placebo with respect to comparability*

| | Diazepam | Placebo |
|---|---|---|
| No of patients | 68 | 68 |
| Age | 15–4? | 14–46 |
| | (27 5) | ( 6 0) |
| Previous induced abortion | 14 | 17 |
| Use of tranquillizers | 12 | 15 |
| Primigravidae | 16 | 18 |

However the same applied to two of the patients who had been given placebo

Assessment of the level of consciousness 1-3 hours after the procedure revealed that 4 patients of the diazepam group and 3 of the placebo group had to be classified as slightly somnolent In all other patients the level of consciousness was unaffected i e they were awake replied adequately and were able to get out of bed unaided

## DISCUSSION AND CONCLUSION

In the present series we have demonstrated that diazepam administered intravenously in a dose of 10 mg significantly prevented the pain of induced abortion performed under local anaesthesia (para cervical block)

We chose a standard dose of 10 mg (1 ampoule) partly for practical reasons and partly because we did not want sedation to be heavier than necessary because of a possible subsequent use of the drug for out patients A dosage per kg body weight would presumably have been more adequate Calculated on the basis of an average body weight of 60 kg the dose used corresponds to 0 17 mg/kg body weight Considering the low toxicity of diazepam (2 4) and the dose of 0 2–0 6 mg/kg body weight suggested by others (8) a somewhat higher dose than ours might presumably have been used with success and without any risk

After abortion on request was permitted in Denmark in 1973 an increased number of abortions may be expected It is desirable therefore to perform induced abortion on out patients or as day

cases partly to avoid a period of waiting and partly not to occupy beds in gynaecological wards un necessarily In this situation it would not be expe dient to sedate the patients too heavily as an in creased dose might be expected to do With the dose used by us the procedure can easily be per formed on an out patient basis as the great majori ty of the patients had a completely unaffected level of consciousness 1–3 hours after the procedure

The amnesia after administration of diazepam which has been reported by others (7 8) was re corded in only about one quarter of our patients possibly because of the lower dose We have no direct explanation why two of the placebo treated patients reported amnesia for the procedure

## ACKNOWLEDGEMENTS

Diazepam (Stesolid®) and placebo were kindly supplied by Dumex Ltd Copenhagen

The Advisory Service of the Danish Medical Research Council rendered assistance in the statistical analysis

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*Submitted for publication Febr 13 1974*

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Acta Obstet Gynec Scand 54 241–246 1975

# STUDIES IN CHOLESTASIS OF PREGNANCY

## III *Fatty Acid Composition of Serum Phosphoglycerides*

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*Abstract* The influence of cholestasis of pregnancy (CP) on liver lipid synthesis as reflected by the composition of serum phosphoglycerides was studied in 28 pregnant women in the last trimester by means of gas-liquid chromatography (GLC) All patients complained of pruritus and had immunologically detectable lipoprotein X (LP X) in serum Twenty women with uncomplicated pregnancies served as a control series In lecithin low palmitic acid (16 0) and high oleic acid (18 1 ($n-9$)) were found which appear to be characteristic for CP The increased oleic acid suggests an enhanced liver lecithin synthesis through the cytidine-diphosphate diglyceride pathway Measurement of the concentrations of lecithin from the gas liquid-chromatograms was made possible by the use of an internal standard fatty acid added which gave a linear relation to direct determination of lecithin For further studies of influences of cholestasis of pregnancy on the relative fatty acid composition lecithin was chosen because differences between lecithin (PC) and phosphoglycerides (GPL) were found evidently due to mutual variations among the three components in GPL (lecithin cephalin and lysolecithin)

Jaundice with a recurrent tendency in subsequent pregnancies has been known for almost 100 years (cf 4) but has been recognized as a clinical entity for only twenty years (11 16) Pruritus gravidarum is a milder form of this complication Morphological studies on liver specimens as well as serum laboratory analyses have revealed changes as in cholestasis (5)

In cholestasis of pregnancy at least two different metabolic influences might be expected those caused by pregnancy itself and those due to cholestasis Variables that might disclose such major metabolic influence are among others the serum lipids through their relative content and specific composition

The lipids in serum are transported as macromolecular compounds the lipoproteins The major lipid constituents of the lipoproteins are free cholesterol cholesterol esters triglycerides and phospholipids which in turn are composed of sphingomyelin and three major phosphoglyceride fractions lecithin (phosphatidyl choline) cephalin (phosphatidyl ethanolamine) and lyso-lecithin

Hormones as well as metabolic products e g bile acids would influence either the synthetic pathways of these lipids in the liver and/or the utilization (turnover) of these lipids in the serum Generally speaking pregnancy as well as estrogen administration give rise to characteristic changes in the serum lipid pattern i e decreased lysolecithin increased lecithin free cholesterol and triglycerides (17) while cholestasis apparently causes an increase in serum free cholesterol and phospholipids through the appearance of a characteristic abnormal lipoprotein the lipoprotein X Data from animal and in vitro studies show that pregnancy and cholestasis can be expected to have more specific influences on the choice of pathways for lecithin synthesis in the liver (2 9 10) The fatty acid distribution of serum phosphoglycerides and particularly lecithin would reflect such metabolic influences

In the present series of papers the fatty acid composition of serum phosphoglycerides and particularly lecithin was analysed in order to elucidate such influences of hormones and bile acids on liver lipid synthesis This paper deals with the fatty acid composition as determined by gas liquid-chromatography in serum phosphoglycerides in cholestasis of pregnancy as compared to normal pregnancy

Table III *Mean differences (Δ) in composition of major fatty acids of phosphoglycerides (GPL) and lecithin (PC=phosphatidyl choline) in control series (normal pregnancies weeks of pregnancy mean 33 6 range 30–37) and series of cholestasis of pregnancy (weeks of pregnancy mean 34 9 range 26–40)*

| | Control series ($n$=20) | | Cholestasis of pregnancy ($n$=28) | |
|---|---|---|---|---|
| | GPL–PC | | GPL–PC | |
| | Δ | *P* | Δ | *P* |
| 16 0 | −2 0 | | −0 5 | – |
| 16 1 ($n$−7) | +0 1 | – | 0 0 | – |
| 18 0 | +0 9 | | +1 6 | |
| 18 1 ($n$−9) | −0 1 | – | +0 3 | |
| 18 2 ($n$−6) | −0 8 | | −1 9 | |
| 20 3 ($n$−6) | 0 0 | – | −0 2 | |
| 20 4 ($n$−6) | +0 6 | | +0 3 | |
| 22 6 ($n$−3) | +0 8 | | +0.3 | – |
| 18–22 ($n$−6) | −0 1 | – | −1 7 | |

Figures are given in mole per cent of methyl esters = 0 05 level =0 01 level =0 001 level

*Distribution among individual phosphatides* In the control series ($n$=20) based on lipid phosphorus determination the mean±S E M values for the relative distribution (in %) among phosphatides showed cephalin (phospatidyl ethanolamine) 6 2±0 4 lecithin (PC) 68 9±0 6 sphingomyelin 19 0±0 5 lysolecithin 4 6±0 3 Expressed only on phosphoglycerides the corresponding relative distribution was cephalin 7 8 lecithin 86 5 and lysolecithin 5 8%

*Relative fatty acid composition of phosphoglycerides (GPL) as compared to that of lecithin (PC)* (Table II Table III) In the *control series* in general the determinations of the relative fatty acid composition showed small variations (evident by small SEM values) both in GPL and in PC When the fatty acid composition of GPL (composed of PC cephalin and lysolecithin) was compared with that of PC alone minor differences were observed In PC 16 0 (palmitic acid) ($p$<0 001) and 18 2 (linoleic acid) ($p$<0 01) were higher while in GPL 18 0 (stearic acid) ($p$<0 001) and 20 4 (arachidonic acid) ($p$<0 01) were higher The latter discrepancy is an expression of the relatively higher content of 18 0 and 20 4 in the cephalin portion of GPL (7 8)

In *the cholestatic pregnancy* the comparison between GPL and PC again revealed in PC a higher content of 18 2 ($p$<0 001) but not in 16 0 As in the control series GPL had a relatively higher content of 18 0 ($p$<0 001) and 20 4 ($p$<0 05) with the same explanation The sum of ($n$−6) was lower ($p$<0 001) in GPL than in PC because the sum of ($n$−6) acids is lower in cephalin (7 8) and in lysolecithin than in lecithin

*Relative fatty acid composition in GPL and PC in cholestatic pregnancy as compared to that in the control series* (Table II Table IV) In the cholestatic pregnancy the variations in the relative fatty acid composition were marked (evidenced by a high SEM value) in 18 2 and sum of ($n$−6) both in GPL and PC The women with cholestasis of pregnancy showed 1) a relatively higher content in 18 1 (oleic acid) in GPL ($p$<0 001) as well as in PC ($p$<0 001) 2) a lower content in 16 0 (palmitic acid) preferentially in PC ($p$<0 001) but also in GPL ($p$<0 05) 3) a higher content of 18 0 (stearic acid) ($p$<0 001) in GPL only and 4) a lower content of 18 2 (linoleic acid) ($p$<0 05) in GPL only

## DISCUSSION

Lecithin (phosphatidyl choline=PC) is the major phosphatide in serum composing in normal pregnancy (30th–37th week) 69% of the total phospholipids or 87% of phosphoglycerides (GPL) The

Table IV *Mean differences (Δ) in composition of major fatty acids of phosphoglycerides (GPL) and lecithin (PC=phosphatidyl choline) in control series (normal pregnancies n=20 weeks of pregnancy mean 33 6 range 30–37) vs series of cholestasis of pregnancy (weeks of pregnancy mean 34 9 range 26–40 n=28)*

| | Control series vs Cholestasis of pregnancy | | Control series vs Cholestasis of pregnancy | |
|---|---|---|---|---|
| | GPL–GPL | | PC–PC | |
| | Δ | *P* | Δ | *P* |
| 16 0 | +1 1 | | +2 7 | |
| 16 1 ($n$−7) | −0 2 | | −0 3 | |
| 18 0 | −1 3 | | −0 6 | – |
| 18 1 ($n$−9) | −2 1 | | −1 7 | |
| 18 ($n$−6) | +1 8 | | +0 6 | – |
| 0 3 ($n$−6) | −0 2 | – | −0 4 | |
| 0 4 ($n$−6) | −0 1 | – | −0 4 | – |
| 6 ($n$−3) | +0 6 | – | +0 1 | – |
| 18– ($n$−6) | +1 4 | | −0 3 | – |

Figures are given in mole per cent of methyl esters = 0 05 level =0 01 level =0 001 level

phosphoglycerides contain in addition cephalin (phosphatidyl ethanolamine=PE) and lysolecithin (lyso phosphatidyl choline) as major components The cephalin and lysolecithin contents constitute 8 and 6% of the phosphoglycerides respectively These latter data of the present study are in agreement with those given by Vikrot (13 17) during normal pregnancy

In the present data differences were found in phosphoglyceride and lecithin fatty acid composition in normal and cholestatic pregnancy The lecithin fatty acid composition has been chosen and will be specifically described and discussed

In cholestasis of pregnancy the lecithin (PC) relative fatty acid content of 16 0 was lower and that of 18 1 higher than during normal pregnancy

Lecithin (as well as cephalin) is esterified by two fatty acids in 1 and 2 position respectively while lysolecithin has only one fatty acid in 1 position The fatty acids in 1 position are preferentially saturated 16 0 and 18 0 while the 2 fatty acids are mono or poly unsaturated 18 1 18 2 and 20 4 (3)

The pathway for synthesis of lecithin in the liver determines the fatty acid composition (3 6 9 10) Lecithin with 16 0 as 1 fatty acid and 18 1 or 18 2 as 2 fatty acid is preferentially synthesized by the cytidine-diphosphate diglyceride pathway Pathway I (9) Pathway II by which methylation of ethanolamine occur in phosphatidyl ethanolamine (cephalin) (10) causes the appearance of a lecithin preferentially with 18 0 in 1 position and 20 4 in 2 position The present data in cholestasis of pregnancy with a lower content of 16 0 and a higher in 18 1 in lecithin would then suggest dual influences by cholestasis on the lecithin synthesis Pathway I A metabolic explanation for these inverse changes in 18 1 and 16 0 in cholestasis of pregnancy is not readily available An increase in 18 1 would suggest an enhanced synthesis of lecithin by Pathway I and would be in agreement with studies in vitro In liver slice technique the addition of bile acids (cholic acid) has been shown to increase the incorporation rate of labelled precursors into Pathway I (2) For the present time the lower 16 0 content in lecithin is recorded as a specific finding in cholestasis of pregnancy

The cholestatic condition causes a decrease of bile acid content in the intestine and would therefore be expected to cause a reduced fat absorption A net decrease in fat absorption would be apparent in those fatty acids not synthesized by the body i e essential fatty acids (EFA) which is indicated by a lower content of sum of $(n-6)$

The use of an internal standard (17 0) in the fatty acid analyses would allow a measurement of lecithin utilizing the obtained formula $y=1\ 66x+75$ (Fig 1) The possibility of measuring lecithin from the fatty acid data would allow a presentation of the quantitative changes in lecithin in cholestasis of pregnancy as compared to normal pregnancy

Utilizing the lecithin amount it would be possible to estimate the absolute value for lecithin with a particular acid From such estimations it was apparent that even on absolute fatty acid composition data the same changes occurred Therefore any metabolic conclusion made in relation to the relative fatty acid composition of lecithin would still be pertinent in relation to the estimated changes in absolute values of lecithin

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| | Control series (n=20) | | Cholestasis of pregnancy (n=28) | |
|---|---|---|---|---|
| | GPL–PC | | GPL–PC | |
| | Δ | P | Δ | P |
| 16 0 | −2 0 | | −0 5 | - |
| 16 1 (n−7) | +0 1 | - | 0 0 | - |
| 18 0 | +0 9 | | +1 6 | |
| 18 1 (n−9) | −0 1 | - | +0 3 | |
| 18 2 (n−6) | −0 8 | | −1 9 | |
| 20 3 (n−6) | 0 0 | - | −0 [illegible] | |
| 20 4 (n−6) | +0 6 | | +0 3 | |
| 22 6 (n−3) | +0 8 | | +0 3 | - |
| 18–22 (n−6) | −0 1 | - | −1 7 | |

Figures are given in mole per cent of methyl esters = 0 05 level =0 01 level =0 001 level

*Distribution among individual phosphatides* In the control series (n=20) based on lipid phosphorus determination the mean±S E M values for the relative distribution (in %) among phosphatides showed cephalin (phospatidyl ethanolamine) 6 2±0 4 lecithin (PC) 68 9±0 6 sphingomyelin 19 0±0 5 and lysolecithin 4 6±0 3 Expressed only on phosphoglycerides the corresponding relative distribution was cephalin 7 8 lecithin 86 5 and lysolecithin 5 8%

*Relative fatty acid composition of phosphoglycerides (GPL) as compared to that of lecithin (PC)* (Table II Table III) In the *control series* in general the determinations of the relative fatty acid composition showed small variations (evident by small SEM values) both in GPL and in PC When the fatty acid composition of GPL (composed of *PC cephalin and lysolecithin*) was *compared with* that of PC alone minor differences were observed In PC 16 0 (palmitic acid) (p<0 001) and 18 2 (linoleic acid) (p<0 01) were higher while in GPL 18 0 (stearic acid) (p<0 001) and 20 4 (arachidonic acid) (p<0 01) were higher The latter discrepancy is an expression of the relatively higher content of *18 0 and 20 4 in the cephalin portion* of GPL (7 8)

In *the cholestatic pregnancy* the comparison between GPL and PC again revealed in PC a higher content of 18 2 (p<0 001) but not in 16 0 As in the control series GPL had a relatively higher content of 18 0 (p<0 001) and 20 4 (p<0 05) with the same explanation The sum of (n−6) was lower (p<0 001) in GPL than in PC because the sum of (n−6) acids is lower in cephalin (7 8) and *in lysolecithin than in lecithin*

*Relative fatty acid composition in GPL and PC in cholestatic pregnancy as compared to that in the control series* (Table II Table IV) In the cholestatic pregnancy the variations in the relative fatty acid composition were marked (evidenced by a high SEM value) in 18 2 and sum of (n−6) both in GPL and PC The women with cholestasis of pregnancy showed 1) a relatively higher content in 18 1 (oleic acid) in GPL (p<0 001) as well as *in PC (p<0 001) 2) a lower content in 16 0* (palmitic acid) preferentially in PC (p<0 001) but also in GPL (p<0 05) 3) a higher content of 18 0 (stearic acid) (p<0 001) in GPL only and 4) a lower content of 18 2 (linoleic acid) (p<0 05) in GPL only

## DISCUSSION

Lecithin (phosphatidyl choline=PC) is the major phosphatide in serum composing in normal pregnancy *(30th–37th week) 69% of the total phospho*lipids or 87% of phosphoglycerides (GPL) The

Table IV *Mean differences (Δ) in composition of major fatty acids of phosphoglycerides (GPL) and lecithin (PC=phosphatidyl choline) in control series (normal pregnancies n=20 weeks of pregnancy mean 33 6 range 30–37) vs series of cholestasis of pregnancy (weeks of pregnancy mean 34 9 range 26–40 n=28)*

| | Control series vs Cholestasis of pregnancy | | Control series vs Cholestasis of pregnancy | |
|---|---|---|---|---|
| | GPL–GPL | | PC–PC | |
| | Δ | P | Δ | P |
| 16 0 | +1 1 | | [illegible] | |
| 16 1 (n−7) | −0 [illegible] | | −0 3 | |
| 18 0 | −1 3 | | −0 6 | - |
| 18 1 (n−9) | − 1 | | −1 7 | |
| 18 2 (n−6) | +1 8 | | +0 6 | - |
| 20 3 (n−6) | −0 | - | −0 4 | |
| 20 4 (n−6) | −0 1 | - | −0 4 | - |
| 22 6 (n−3) | +0 6 | - | +0 1 | - |
| 18–22 (n−6) | +1 4 | | −0 3 | - |

Figures are given in mole per cent of methyl esters = 0 05 level =0 01 level =0 001 level

Acta Obstet Gynec Scand 54 247–250 1975

# UTERINE SIZE MEASURED BY ULTRASOUND DURING THE MENSTRUAL CYCLE

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*Abstract* In the following study changes in the size of non pregnant uterus were measured by B-scan ultrasonography Uterine size in different stages of the menstrual cycle was measured ultrasonically in 16 women whose periods were confirmed to be ovulatory both by basal body temperature (BBT) and by the radioimmunological measurement of plasma estradiol and progesterone It was established that the size of the uterus grows significantly towards the end of the menstrual cycle In addition to the above mentioned the examination was performed on a woman of child bearing age with disturbances in her menstrual cycle Hormone measurements indicated that her period was anovulatory No typical uterine growth characteristic of ovulatory cycles could be observed

In gynecological treatment and research it is often necessary to know the size of the uterus Uterine size as determined by a gynecological examination even under the most favourable circumstances is only an approximate estimate An objective method of measurement is desirable

In his previous study Puroinen (5) has proved that B scan ultrasonography which is used in estimating uterine size during pregnancy is also reliable in measuring the size of a non pregnant uterus It was observed that the uterus of a woman of child bearing age grew with the number of births No correlation was established in examining the effect of age on uterine size in the same parity group

The purpose of this study has been to examine the possible effect of the stage of the menstrual cycle on uterine size This was carried out by following the same uterus in different phases of the cycle with ultrasonic B scanning

## MATERIAL AND METHODS

For the purpose of this study we chose 16 healthy women with a regular 28 day menstrual cycle Four of these women were parous and 12 non-parous Their ages varied from 19 to 41 years An attempt was made to examine each woman by ultrasound on the 5th 12th 21st and 27th day of the cycle with ± one day accuracy The menstrual cycle of three women proved to be longer than supposed 32–34 days so an extra ultrasound examination was performed before the beginning of menstruation In three other cases the cycle lasted only 25–26 days so the last examination had to be carried out at the onset of menstruation The participants measured their BBT during the examination period In addition ovulation was confirmed by measuring at the same time as the ultrasound examinations plasma progesterone (2) and estradiol (4) by radio-immunological methods It was presumed that the level of progesterone in the proliferative phase was <5 nmol/l and in the luteal phase>15 nmol/l On the 12th day of the cycle an attempt was made to ascertain the estradiol peak and in the luteal phase the rise of the estradiol value to at least 0 30 nmol/l (1) Besides these studies were made on a 29 year-old parous woman who had had menstrual disturbances for the previous 6 months

The ultrasound examination was performed with Kretz technik ultrasound equipment (4100 MG S) using a 2 megacycle probe olive oil as contact medium and the full bladder technique The aim was to have as nearly as possible an equally full bladder in the same person at each examination Ten of those being examined attended to the filling of the bladder their own by drinking and abstaining from passing urine The degree of fullness of the bladder was ascertained by B-scanning

Before the ultrasonic scanning the bladders of six women were emptied by catheter after which 400 ml of 0 9% NaCl solution was run into the bladder via the catheter B-scanning was begun with a scale size of 1 2 An attempt was made to find the uterine midline by taking several longitudinal cross-sections After it had been found the ultrasound equipment was set to the biggest scale value (1 1) and the uterine longitudinal midline section was photographed from the memory scope Following this the examination was repeated The area of the uterine longitudinal midline section was measured with a planimeter from both photographs obtained and the average of results was calculated

## RESULTS

Fig 1 shows the longitudinal midline section of a uterus on the 5th 12th 21st and 27th day of the

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*Submitted for publication Febr 19 1974*

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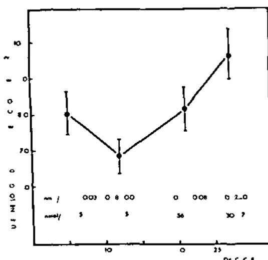


*Fig 3* The area of the uterine longitudinal midline section (mean ± S E M ) in different stages of the menstrual cycle in 10 women whose period lasted 28 days The estradiol ($E_2$) and progesterone (P) values (mean ± S E M ) obtained during the examinations are indicated in the figure

## DISCUSSION

Before ultrasound diagnosis was introduced into gynecology it was difficult to examine the size of a uterus reliably In his previous work Piiroinen (5) has proved that the length and the biggest AP measurement can be reliably obtained from the longitudinal midline section of a non pregnant uterus The length and the AP measurement of 20 uteri were established by ultrasound before removal of the uterus and mechanically immediately after the operation The measurements obtained by ultrasound did not differ from those obtained mechanically (length $p=0\ 20$ and AP reading $p=0\ 05$)

The present study indicates that it is possible to establish changes in the size of a non pregnant uterus by B scanning Uterine size was determined by measuring the area of the longitudinal midline section In order to estimate the reliability of the method each woman was examined two consecutive times When the results of these examinations were compared using the difference $t$ test no difference could be proved ($t=0\ 94$ $f=54$ $p>0\ 30$) The coefficient of variation calculated from all duplicate determinations was ± 6 3 %

The ultrasonic examination of a non pregnant uterus requires a full but not over stretched bladder A too-full bladder exerts pressure on the uterus towards the spine and can change the shape of the uterus The amount of urine in the bladder can be measured with a 20 % accuracy with ultrasound (3) The study indicated that the women being examined were each able to estimate quite accurately the fullness of the bladder If there is sufficient time and the examinees are co-operative catheterization is not necessary to obtain the same fullness of the bladder in repeated examinations of the same person Retroversion of the uterus (one case) was not found to disturb the examination

Uterine size varies individually and grows with parity In this study the original size of the uterus did not have any effect on growth occurring during the menstrual cycle In comparing the results of the 12th and 27th days the area of the uterine longitudinal midline section grew 1 36±0 05 times (mean ±S E M ) If the uterus is presumed to be a ball thus signifies that the volume grows 1 6 times on the average If th. ultrasonic scanning could have been performed on each day of the menstrual cycle it is possible that the smallest uterine size would have

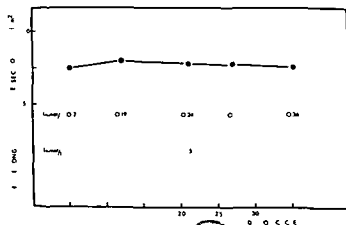


*Fig 4* The area of the uterine longitudinal midline section and the corresponding estradiol (E ) and progesterone (P) values of an anovulatory cycle

been found in the proliferative phase already before the 12th day of the cycle In that case the difference between the size of the biggest and smallest uterus would be even more marked

It is known that estrogen and progesterone cause cyclical changes in the uterus during a normal menstrual cycle Estrogen induces a sequence of metabolic events which lead to an increase in the size of the uterus These well known changes like the increase of RNA and proteinsynthesis the rise of enzyme activity and changes in membrane permeability lead to hypertrophy and hyperplasia of the uterus In this series no correlations were observed between estradiol and progesterone values or proportions of these and the amount of uterine growth Cyclical changes in the size of the uterus however imply hormonal stimulus In the anovulatory cycle under examination a lack of both hormonal stimulus and typical uterine growth was established

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*Submitted for publication Febr ?5 1974*

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Acta Obstet Gynec Scand 54 251–253 1975

# ACID-BASE RELATIONSHIP BETWEEN MOTHER AND FETUS IN GESTOSIS (PRE ECLAMPSIA) AND IN PREGNANT WOMEN WITH A LABILE BLOOD PRESSURE

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*Abstract* Simultaneous blood microsamples were taken from the maternal ear and uterine cervix and the fetus of healthy pregnant women those with labile hypertension and those with severe gestosis (pre-eclampsia) The $p_{O_2}$ $p_H$ BE and $p_{CO_2}$ were studied The fetuses showed no signs of asphyxia The differences in BE values between the women with gestosis and their fetuses were significantly greater than in the cases of healthy pregnancy No differences were seen between healthy and gestotic gravida groups in $p_{O_2}$ $p_H$ and $p_{CO_2}$ values This favours the opinion that in gestosis of the mother the fetus has a tendency to metabolic acidosis which apparently places it in a poorer position than the fetuses of healthy mothers should acute asphyxia occur At the time of study the $p_{O_2}$ of the fetuses of gravidas with a labile blood pressure was lower than that of fetuses of healthy gravidas

An impaired exchange of gases between mother and fetus is frequently observed in gestosis of pregnancy (pre-eclampsia) as a consequence of impaired uterine and placental perfusion (2 4 5) Clinically this condition manifests itself in retarded development and poorer postnatal condition of the infants of mothers with gestosis as compared with those of healthy mothers (8) This is often the case even if no signs of asphyxia have been observed during pregnancy The possible reflection of this circumstance in the maternal and fetal Astrup values was investigated in the study reported here

## MATERIAL AND METHOD

Simultaneous blood microsamples were taken from the maternal ear and cervix and the fetal scalp in 16 cases of healthy pregnancy 6 mothers with gestosis and 11 with labile blood pressure of late pregnancy The blood pressure of the healthy mothers was below 140/90 and the Esbach test showed no proteinuria The patients with gestosis had blood pressure over 170/115 and proteinuria of 0 4 g/day or more Edema was not recorded If during the last week of pregnancy the mother had a few blood pressure readings above 140/90 while the pressure in general was normal and the urine free from protein she was included in the labile hypertensives group All the subjects were in the 36th–40th week of pregnancy the membranes were ruptured spontaneously or artificially and labour had not become established The fetuses showed no signs of asphyxia

The $p_{O_2}$ value was determined from the blood sample taken from the ear In addition to $p_{O_2}$ also $p_H$ BE and $p_{CO_2}$ were determined from the other blood samples

## RESULTS

The results obtained are presented in absolute values in Table I No significant differences were found when the values for healthy mothers were compared with those with gestosis On the other hand comparison of the healthy subjects with those having a labile blood pressure showed a significant difference in the fetal $p_{O_2}$ values This value was 29 2 mmHg in the former group and 25 5 mmHg in the latter ($t$=3 03 $p$<0 01)

Additionally a comparison was made between the differences in the maternal and fetal values in the three groups The results of comparison are seen in Table II Significant differences between the groups were not found in $p_{O_2}$ $p_H$ or $p_{CO_2}$ The difference between the cervical and fetal BE values in the gestosis group differed significantly from the difference in the normal pregnancy group the latter being 1 16 mEq/l and the former 3 10 mEq/l ($t$=2 12 $p$<0 05)

Table I *Simultaneous maternal and fetal Astrup values in mothers with gestosis labile hypertension and normal pregnancy (controls)*

| | No | $p_{O_2}$ mmHg×100 | | | $p_H$ | | BE mEq/l | | $p_{CO_2}$ mmHg | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Ear | Cervix | Fetus | Cervix | Fetus | Cervix | Fetus | Cervix | Fetus |
| Controls | 16 | 101 8 | 73 2 | 29 2 | 7 406 | 7 377 | −4 47 | −5 68 | 30 47 | 37 15 |
| Gestosis | 6 | 103 2 | 76 5 | 29 2 | 7 427 | 7 323 | −4 87 | −7 97 | 26 08 | 31 9[illegible] |
| Labile hypertension | 11 | 95 8 | 74 2 | 25 5 | 7 408 | 7 319 | −6 43 | −7 14 | 2[illegible] 08 | 34 3 |

## DISCUSSION

The fetal $p_{O_2}$ values in mothers with a labile blood pressure were lower than those in healthy gravidas These persons evidently suffered from a vasomotor defect which also was reflected in their tenseness during the test The finding can be considered an indication of the large role that external factors may have in for example the oxygen supply to the fetus

A very good correlation has been found to exist between the base deficit (negative base excess) of healthy mothers and their fetuses (1 3 6) When the fetus becomes asphyxiated lactic acid is generated and there is a decrease in the base excess of the fetus relative to that present in the This difference has proved useful in de ing the significance of a low fetal $p_H$ and is a decreased base excess has also been found to correlate with the Apgar score (1)

In the earlier studies the maternal sample was taken from the cubital vein and not from the uterine cervix as in the present work In gestosis the impaired blood circulation of the uterine wall is already reflected also in the cervical blood values Despite this a greater difference between the maternal and fetal BE values was seen in the gestosis group than in the healthy group Therefore it apparently is a question of deficient blood circulation of the placenta

In gestosis the exchanges of gases between mother and fetus remain at a constant low level for a long time During this time there is compensation of the pathological $p_H$ and $p_{CO_2}$ values that occur in acute fetal asphyxia On the other hand there will be an increase in the difference of maternal and fetal BE denoting metabolic acidosis resulting from a disturbance of long duration

Even in the absence of clinical signs of asphyxia the fetuses of mothers with gestosis apparently have often had to adjust their metabolism to an economic level (7) As a result they have smaller reserves in the possible event of acute asphyxia

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Table II *Differences in Astrup values between ear cervix and fetal blood in mothers with gestosis labile hypertension and normal pregnancy (controls)*

| | No | $p_{O_2}$ mmHg | | | $p_H$ | BE mEq/l | $p_{CO_2}$ mmHg |
|---|---|---|---|---|---|---|---|
| | | Ear cervix | Ear–fetus | Cervix–fetus | Cervix–fetus | Cervix–fetus | Cervix–fetus |
| Controls | 16 | 23 0 | 72 6 | 44 7 | 0 089 | −1 16 | −9 06 |
| Gestosis | 6 | 26 7 | 74 0 | 47 3 | 0 103 | −3 10 | −5 83 |
| Labile hypertension | 11 | 21 5 | 71 3 | 48 3 | 0 084 | −0 71 | −7 36 |

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*Submitted for pi blicati in Febr ?6 1974*

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Acta Obstet Gynec Scand 54 255–259 1975

# DYSMENORRHEA IN INDUSTRIAL WORKERS

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*Abstract* In two Norwegian industrial companies 234 women of menstruating age were examined by the industrial nurse with regard to menstrual complaints Every second woman experienced pain 23% had consulted a doctor previously about 30% had to stay in bed and about 30% had been absent from work recently due to dysmenorrhea Although pain was prevalent in all age groups there were age specific differences in other complaints such as headache and depression which were more frequent among the older women In a selected group of 32 women with severe complaints the history and gynecological examination indicated secondary dysmenorrhea in only a few cases Hormonal assays and endometrial biopsy indicated anovulatory cycles in 4 out of 12 women in spite of dysmenorrhea

Dysmenorrhea is one of the commonest disorders in women The frequency varies in different reports probably due to differences in the population groups studied In Finland 13 2% of school girls had regularly recurring dysmenorrhea (5) Nine years after the menarche over 26% of girls had dysmenorrhea In a study from Sweden Svennerud (4) found that 31% of 890 women aged 14 to 44 had dysmenorrhea In almost half of the group the pain was sometimes or regularly disabling

The pain of primary dysmenorrhea may be so severe that the woman must stay in bed being unable to perform her daily duties Since this periodic disorder however incapacitating is often regarded as a normal phenomenon many cases do not come to the doctor s attention The cause of this disorder is still uncertain Many forms of treatment have been tried with varying results Prophylactic treatment is possible through the use of steroid hormones which are not always accepted by the patients Physiotherapy has also been reported to be of benefit (?)

In some Norwegian industrial companies with many young female employees periodic inability to work due to menstrual disorders has been a problem of some magnitude and the question has been raised whether such absence is medically justified in view of modern hormonal treatment In this connection it is interesting to note that Svennerud (4) found the frequency of dysmenorrhea to be the same in women of widely different occupations such as factory workers actors and gymnasts Furthermore the absence from work due to dysmenorrhea in factory workers represented only 3 7% of absence from all causes In Norway no previous study has been aimed specifically at menstrual disorders as a cause of work incapacitation Therefore the Industrial Health Service Council (Bedriftslegerådet) in Norway in 1970 initiated the following epidemiological and gynecological study (1 3)

To approach the problem we planned to examine the occurrence of menstrual disorders particularly dysmenorrhea among female employees in some Norwegian companies in which this appeared to be a problem The intention was to interview all the female employees and to refer those who had the most distressing symptoms to a gynecologist for examination and treatment

## MATERIAL AND METHODS

The subjects examined were employed in an electrical/technical company in Oslo and in a textile industry in Bergen A questionnaire provided by the industrial nurse was completed by women of menstruating age who were seen for pre-employment or periodic medical examinations in 1970 to 1971 The questionnaire included questions on previous operations obstetrical history regularity of menstruation headache depression polymenorrhea premen

Table I *The absolute and relative age distribution within the two companies of the primary series*

| Material | | Age (years) ≤19 | 20–29 | 30–39 | 40–49 | ≥50 | Total |
|---|---|---|---|---|---|---|---|
| Oslo (electrical/ technical company) | No | 28 | 66 | 14 | 23 | 4 | 135 |
| | % | 20.7 | 48.9 | 10.4 | 17.1 | 2.9 | 100.0 |
| Bergen (textile industry) | No | 26 | 61 | 10 | 2 | – | 99 |
| | % | 26.3 | 61.7 | 10.0 | 2.0 | – | 100.0 |
| Total | | 54 | 127 | 24 | 25 | 4 | 234 |

strual weight increase, previous treatment including usage of contraceptive pills and absence from work due to dysmenorrhea. On the basis of individual symptoms and absence from work due to dysmenorrhea in the sickleave records subjects who were considered to suffer from severe dysmenorrhea were selected for closer examination by a gynecologist (H. J. in Oslo and P. B. in Bergen). This included a more detailed medical history followed by an ordinary gynecological examination, including cytological smear, and in some cases urinary and serum assays of hormonal steroids. Based on the clinical findings, the dysmenorrhea was classified as primary or secondary. Treatment and follow-up examinations on the effect of treatment were also offered.

Table II *The occurrence of complaints with relation to menstruation in the primary series*

| Complaints | Oslo No | Oslo % | Bergen No | Bergen % | Combined Oslo/Bergen No | Combined Oslo/Bergen % |
|---|---|---|---|---|---|---|
| Dysmenorrhea[a] | 89 | 66 | 53 | 54 | 142 | 61 |
| Pain | 69 | 51 | 47 | 47 | 116 | 50 |
| Headache | 33 | 24 | 2 | 2 | 35 | 15 |
| Feeling unwell | 24 | 18 | 14 | 14 | 38 | 16 |
| Depression | 44 | 33 | 4 | 4 | 48 | 21 |
| Polymenorrhea | 24 | 18 | 7 | 7 | 31 | 13 |
| Other complaints | 15 | 11 | 5 | 5 | 0 | 9 |
| Weight increase | 25 | 19 | 12 | 12 | 37 | 16 |
| Treatment necessary (analgesics and/or hot water bag) | 49 | 36 | 42 | 42 | 91 | 39 |
| Confinement to bed necessary | 36 | 27 | 29 | 29 | 65 | 8 |
| Absence from work due to dysmenorrhea (last six months) | 44 | 33 | 28 | 28 | 72 | 31 |
| Previous medical consultation for dysmenorrhea | 32 | 24 | 22 | 22 | 54 | 23 |
| All subjects | 135 | 100 | 99 | 100 | 234 | 100 |

[a] Dysmenorrhea = Pain and/or other complaints shortly before or during menstruation last three months.

Altogether 234 women were examined. The age distribution within the two companies is shown in Table I. Of these 60 with severe dysmenorrhea were selected for a gynecological examination, but only 32 were actually seen by the gynecologists. Failure to attend was for various reasons, such as neglect, unwillingness to come or leaving the job.

## RESULTS

### *The primary series*

The occurrence of the various complaints related to menstruation is presented in Table II. Dysmenorrhea, defined as pain and/or other complaints shortly before or during menstruation during the last three months, was recorded in 61% of the combined Oslo/Bergen series. The difference in prevalence between Oslo and Bergen was small. In the dysmenorrhea group 77% were nulliparous which was of the same order as found in subjects without dysmenorrhea (80%). With regard to several specified parameters, such as pain, feeling unwell, treatment necessary, confinement to bed, absence from work and medical consultation, a similar frequency in the Oslo and the Bergen groups was observed. Every second woman experienced pain during menstruation and 28% had to stay in bed. Absence from work was noted in 31% and 23% had previously consulted a doctor for dysmenorrhea.

A striking discrepancy, however, was found with regard to headache (Oslo 24% versus Bergen 2%) and depression (Oslo 33% versus Bergen 4%). There was slight but noticeable difference for polymenorrhea (Oslo 18% versus Bergen 7%). Thus, all these three parameters were observed with a *higher frequency* in Oslo than in Bergen. The differences in prevalence between Oslo and Bergen regarding the three symptoms are most certainly caused by the age difference between the two groups (see below).

Table III *The occurrence of complaints with relation to menstruation in the combined Oslo/Bergen material according to age*

| | Age (years) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ≤19 | | 20–29 | | 30–39 | | 40–49 | | ≥50 | | Total | |
| Complaints | No | % | No | % | No | % | No | % | No | % | No | % |
| Dysmenorrhea | 28 | 52 | 75 | 59 | 14 | 58 | 21 | 84 | 4 | – | 142 | 61 |
| Pain | 28 | 5 | 66 | 52 | 9 | 38 | 12 | 48 | 1 | – | 116 | 50 |
| Headache | 4 | – | 13 | 10 | 8 | 33 | 10 | 40 | – | – | 35 | 15 |
| Feeling unwell | 12 | 22 | 19 | 15 | 3 | – | 4 | – | – | – | 38 | 16 |
| Depression | 2 | – | 26 | 20 | 6 | 25 | 12 | 48 | 2 | – | 48 | 21 |
| Polymenorrhea | 4 | – | 15 | 12 | 2 | – | 8 | 32 | 2 | – | 31 | 13 |
| *Other complaints* | 2 | – | 9 | 7 | 2 | – | 5 | – | 2 | – | 20 | 9 |
| Weight increase | 2 | – | 23 | 18 | 3 | – | 8 | 32 | 1 | – | 37 | 16 |
| Treatment necessary (analgesics and/or hot water bag) | 19 | 35 | 57 | 45 | 7 | 29 | 8 | 32 | – | – | 91 | 39 |
| Confinement to bed necessary | 17 | 31 | 40 | 31 | 4 | – | 3 | – | 1 | – | 65 | 28 |
| Absence from work due to dysmenorrhea (last six months) | 13 | 24 | 45 | 35 | 6 | 25 | 7 | 28 | 1 | – | 72 | 31 |
| Previous medical consultation for dysmenorrhea | 11 | 20 | 28 | 22 | 4 | – | 10 | 40 | 1 | – | 54 | 23 |
| *All subjects* | *54* | *100* | *127* | *100* | *24* | *100* | *25* | *100* | *4* | *100* | *234* | *100* |

The frequency (%) has not been calculated if the number in the group is ≤5

The age specific rates of the various complaints are also given for the combined Oslo/Bergen series (Table III) The prevalence of dysmenorrhea was almost constant about 55 % up to the age of 40 After that age the prevalence increased to 84 % in the age group 40–49 and all four women aged 50 and over complained of dysmenorrhea Among complaints pain was recorded with the highest frequency the prevalence showed some irregular fluctuations with age (38–52 %) A similar pattern was found for those who needed treatment (29–45 %) and those who had been absent from work (24–35 %) For three parameters there was a noticeable increase with age headache (10–40 %) depression (20–48 %) and previous medical consultation for dysmenorrhea (20–40 %)

Furthermore it should be mentioned that in the primary series menstruation was regular in 82 % and the usage of contraceptive pills was reported in 9 %

### *Patients with severe dysmenorrhea seen by the gynecologists*

Of the 32 patients seen by the gynecologists 19 had sedentary work while 11 had a job requiring a moderate or great amount of physical activity The character of their work did not seem to influence the kind of complaint Twenty five were nulliparous (78 %)

Fifteen gave a history of previous gynecological disease (Table IV) The mean age at menarche was 12 8 years The interval from menarche to start of dysmenorrhea is shown in Table V

The duration of dysmenorrhea is shown in Table VI In Oslo the complaints appeared to be of slightly shorter duration than in Bergen Ten women suffered from other symptoms than pelvic pain during menstruation (backache dysuria nausea vomiting), while 5 gave a history of premenstrual tension

Table IV *Previous gynecological disease in women with severe dysmenorrhea*

| Disease | No of patients | Total |
|---|---|---|
| Salpingitis | 4 | 15 |
| Menometrorrhagia | 4 | |
| Ovarian resection | 1 | |
| Endometriosis laparotomy | 1 | |
| Vaginitis | 4 | |
| Gonorrhea | 1 | |
| No gynecological disease | 17 | 17 |
| Total | | 32 |

Table V *Interval from menarche to start of dysmenorrhea in women with severe dysmenorrhea*

| Interval (years) | No of patients |
|---|---|
| No interval | 8 |
| 1–2 | 9 |
| 3–5 | 6 |
| 6–11 | 4 |
| Uncertain | 5 |
| Total | 32 |

dyspareunia and 2 of midcycle pelvic pain Nineteen were confined to bed one day or more during every menstrual period 7 others were unable to attend their job while 6 occasionally had to be absent from work because of the dysmenorrhea

The dysmenorrhea was of constant severity in 17 cases varying from cycle to cycle in 10 cases of increasing severity in 4 and decreasing in 1 case

Twenty-eight of the patients had received some form of medical treatment previously analgesics contraceptive pill gestagens or cervical dilatation Vaginal examination showed excessive discharge in 4 patients cervical ectopy in 4 cases a small ovarian cyst in 2 cases a tender ovary located in the pouch of Douglas in one case and a thickening of the parametrium in one case

The uterus was considered to be small in 8 cases one patient had uterine fibromyomata Extreme inteflexion of the uterine body was found in one case retroversion in another case

On the basis of hormonal assays of plasma progesterone or urinary pregnanediol and premenstrual endometrial curettage performed in 12 women 4 were considered to have an anovulatory cycle in spite of the dysmenorrhea

The combined estrogen/progestogen contraceptive pill was given to 10 patients who accepted this form of treatment Only 5 were controlled and all of these reported excellent pain relief Of 4 patients receiving dehydroretroprogesterone only one was controlled and she too reported effective pain relief A combined analgesic pill composed of dextropropoxyphen phenacetin and acetylsalicylic acid was given to two patients and was ineffective in both

## DISCUSSION

The investigation revealed a very high prevalence of dysmenorrhea among industrial workers Every second woman complained of pain and when all complaints are considered 61% had monthly complaints which must obviously affect their capacity for work As 9% of the women were using contraceptive pills which reduce or eliminate dysmenorrhea the true prevalence may even be slightly higher About 30% of the women were confined to bed About 30% had been absent from work which illustrates the magnitude of the problem both medically and economically

The prevalence of dysmenorrhea is higher than that reported by others (4 5) even if the complaint is restricted to pain This may be due to the definition of the disorder A certain degree of pain which does not interfere with the daily duties may have been regarded as normal in other investigations Age differences in different series may also play a part The fact that the prevalence of the major complaints was almost equal in the Oslo and Bergen companies indicates that the figures are not caused by interview bias

Dysmenorrhea is often regarded as a complaint which recedes with age It is therefore of interest to note that with the exception of the age group 30-39 the prevalence of pain was about the same in all the other groups up to the age of 49 Considering the small number of subjects in the higher age groups a few cases of secondary dysmenorrhea will mean a considerable increase in frequency The pattern of other complaints associated with menstruation changed with age Not unexpectedly the prevalence of headache depression and polymenorrhea was higher in the older age groups To a large extent this is thought to be a reflection of changing ovarian function The differences in prevalence between Oslo and Bergen regarding the three symptoms mentioned are most certainly caused by the age difference between the two groups There were more women in the higher age groups in the Oslo company

Of 12 women who were examined by endometrial

Table VI *Duration of pain (days) in women with severe dysmenorrhea*

| Days | 1 | 2 | 3 | 4 | 5 | Total |
|---|---|---|---|---|---|---|
| Oslo | 10 | 4 | 3 | 1 | 0 | 18 |
| Bergen | 3 | 6 | 0 | 1 | 4 | 14 |
| Total | 13 | 10 | 3 | 2 | 4 | 32 |

curettage or hormonal assay about one week prior to menstruation 4 were thought to have anovulatory cycles Anovulatory cycles are not uncommon particularly in the first few years after the menarche Dysmenorrhea is often regarded as a sign of preceding ovulation but this may be misleading

Treatment should be individualized Prophylaxis with pelvic exercises was not tried as a part of the present investigation Although dysmenorrhea was found to affect women with sedentary work and those with manual work similarly physical exercise aimed specifically at strengthening the pelvic muscles and increasing the pelvic blood supply ought to be tried (2) Estrogen containing contraceptive pills are effective in most cases but it is our experience that many young women simply refuse to take contraceptive pills as treatment for dysmenorrhea The same is true of pure progestational steroids which in Norway are fairly expensive when taken for three weeks each month We feel that if a woman prefers to have monthly discomfort to the daily ingestion of a hormonal pill which may cause side effects this should be her own choice even if it means one or two days absence from work Dysmenorrhea is only a minor cause of absence among women when the total number of lost working days are considered

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*Submitted for publication March 13 1974*

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Acta Obstet Gynec Scand 54 261–264 1975

# THE SIGNIFICANCE OF ORAL CONTRACEPTIVES IN CAUSING CHROMOSOME ANOMALIES IN SPONTANEOUS ABORTIONS

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*Abstract* The significance of oral contraceptives in causing chromosome aberrations in the fetus was studied in 246 non selected spontaneous abortuses using Q-banding technique No significant difference in the frequency of abnormal karyotypes or in the sex ratio was found between 124 abortuses of women who had taken oral contraceptives in comparison with 122 abortuses of women who had never used oral contraceptives The study did however show that women who had used oral contraceptives were significantly younger than women who had not used these pills In addition the gestational age of the chromosomally abnormal abortuses was on the average 6 days longer in the group of women who had used oral contraceptives than in the group who had not The difference was significant only with regard to the karyotype 45 X

No definitive answer has yet been given to the question whether oral contraceptives (OC) might induce chromosome aberrations

Some authors have found an increased number of chromosome breaks in lymphocyte cultures from women taking OC (8 9) These findings have been questioned by others who failed to demonstrate any significant change in the frequency of chromosome aberrations in lymphocyte cultures from women prior to and after long term use of OC (6 10) Spontaneous abortuses from women previously using OC have been reported to have an increased frequency of chromosome anomalies (5 1) for triploidy the increased frequency was significant (3)

In the present work an attempt was made to elucidate the problem by comparing spontaneous abortuses from women previously having taken OC with abortuses from women who had never used OC

## MATERIAL AND METHODS

A total of 286 consecutively collected aborted fetuses were studied (11) They comprised all spontaneous abortuses from a single hospital during the period April 1971 – May 1973 Five abortuses from women who had been treated with gonadotrophic hormones immediately before pregnancy and three from women who became pregnant despite the use of intrauterine contraceptive devices were excluded Chromosome analysis was performed on cells from long term cultures Successful growth was obtained in 254 out of 287 cases i e 88 5% Karyotypes were made of cells stained with orcein in all cases In addition all abnormal and most normal karyotypes were analysed by the Q-banding technique The patients were questioned about the use of OC immediately before discharge from the hospital The information obtained was checked approximately four weeks later in association with a follow-up examination of both parents at the out patient clinic of the hospital All tissue samples were collected and all interviews were undertaken by the author A list of the different contraceptives used by the women in the present study and the mean duration of use is given in Table I Of the 124 women who had taken OC 44% had used more than one preparation.

## RESULTS

*Cytogenetic analysis*

The results of the chromosome analysis are given in Table II Sixty per cent of the abortuses of mothers who had taken OC revealed a chromosome abnormality The corresponding figure for mothers who had never taken OC was 49% The difference is not significant ($\chi^2_1$=3 17 0 05<$P$<0 1) Furthermore there was no significant difference between mothers who had taken and those who had not taken OC with regard to the frequency of different types of chromosome aberrations

There was a marked difference in age between the women who had used and those who had not used oral contraceptives (Fig 1) The mean age of mothers who had an abortus with a normal karyotype

Table I *The contraceptives used by the women included in the present study*

| Type of contraceptive | Mean duration of contra ceptives (months) | No of pats |
|---|---|---|
| Norgestrel 0 5 mg + Ethinyl estradiol 0 05 mg | 24 2 | 25 |
| Ethynodiolacetate 1 mg + Mestranol 0 1 mg | 29 5 | 13 |
| Megestrol acetate 4 mg + Mestranol 0 05 mg | 26 9 | 11 |
| Medroxiprogesterone acetate 10 mg + Ethinyl estradiol 0 05 mg | 19 0 | 3 |
| Lynestrenol 2 5 mg + Mestranol 0 075 mg | 30 2 | 15 |
| Norethisterone acetate 3 mg + Ethinylestradiol 0 05 mg | 28 5 | 2 |
| Used two or more contra ceptives | 24 6 | 55 |
| Total | 25 9 | 124 |
| | (Range 2–60 months) | |

and had used OC was 24 7 (±3 1) years as compared with 28 7 (±5 1) years for those who had not used OC The corresponding mean ages for mothers who had an abortus with an abnormal karyotype were 26 0 (±4 2) and 28 3 (±6 0) years respectively The difference between these age distributions is highly significant ($\chi^2_4$ =26 9 $P$<0 0005) However there

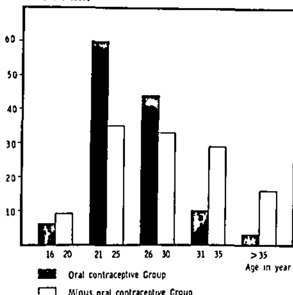



*Fig 1* Maternal age distribution of 246 cases of spontane ous abortions

Table II *Results of the cytogenetic analysis of 246 abortuses*

| Karyotype | Oral contra ceptives No | Oral contra ceptives % | Minus oral contraceptives No | Minus oral contraceptives % |
|---|---|---|---|---|
| 46,XY | 22 | | 31 | |
| 46 XX | 27 | | 31 | |
| Total normal | 49 | 40 | 62 | 51 |
| Tetraploidy | 4 | | 7 | |
| Triploidy | 8 | | 6 | |
| 45 X | 23 | | 15 | |
| Trisomy 2 | | | 1 | |
| 4 | 1 | | 1 | |
| 7 | 1 | | 1 | |
| 8 | 3 | | | |
| 9 | | | 3 | |
| 10 | 1 | | 1 | |
| 13 | | | 1 | |
| 14 | 4 | | | |
| 15 | 6 | | 4 | |
| 16 | 9 | | 11 | |
| 18 | 4 | | 2 | |
| E | | | 1 | |
| 21 | 2 | | 2 | |
| 22 | 1 | | | |
| C or G | 1 | | | |
| X | 1 | | | |
| Double trisomy | | | 2 | |
| Mosaics and transloca tions | 6 | | 2 | |
| Total abnormal | 75 | 60 | 60 | 49 |

was no difference within the individual age groups in the frequency of chromosome abnormalities be tween those who had used and those who had not used OC

### *Sex ratio*

The ratio between the number of abortuses having a Y chromosome and those lacking this chromosome was the same in the group in which the mothers had used OC as in the one in which they had not Table II shows the sex distribution for the normal karyotypes Among those with an abnormal kar yotype there were 26 males and 24 females in the OC group and 22 males and 24 females in the other The 45 X cases were excluded in this calculation

### *Gestational age*

The mean gestational age i e the time from the first day of the last menstruate period until abortion minus 14 days is given in Table III for groups of abortions divided according to karyotype The ges tational age for chromosomally abnormal abortions

was longer in the OC group than in the other but the difference was only significant for abortuses with 45 X karyotype (Mann Whitney test $P<0.01$)

*Interval between stopping OC and pregnancy*

The time from stopping OC to the beginning of the present pregnancy is given in Table IV for abortions with normal and abnormal karyotypes respectively No significant differences were noted ($\chi^2_2 = 2.21$ $0.3<P<0.4$)

## DISCUSSION

The problem whether oral contraceptives might induce chromosome abnormalities is most efficiently investigated in spontaneous abortions since most fetuses with gross chromosome aberrations never go to term

In the present series of 246 consecutively collected spontaneous abortuses no significant difference was found between the frequency of chromosome abnormalities among abortuses from mothers who had used OC before conception compared with that from mothers who had never used OC This was the case even in women who had become pregnant within 6 months after stopping the pills The mean age of the women who had used OC was on the average 3 years lower than the mean age of women who had never used OC The difference is significant ($P<0.0005$) and might be a source of error in a study like the present one since the origin of trisomy 21 as well as of trisomy 13 and 15 is dependent on the maternal age at conception (7)

Table III *Distribution of abortuses according to gestational age*

| Karyotype | Mean gestational age in days | |
|---|---|---|
| | Oral contraceptives | Minus oral contraceptives |
| 46 XY | 70 83 | 74 77 |
| 46 XX | 72 60 | 70 42 |
| Total normal | 71 80 | 7[illegible] 60 |
| Tetraploidy | 6[illegible] 50 | 6[illegible] [illegible]9 |
| Triploidy | 76 43 | 68 50 |
| 45 X | 78 78 | 69 00 |
| Trisomy | 67 65 | 66 48 |
| Others | 69 14 | [illegible]8 75 |
| Total abnormal | 71 86 | 66 31 |

Table IV *Interval between stopping oral contraceptives and pregnancy*

| Interval (mo) | Abortuses with normal karyotypes (no) | Abortuses with abnormal karyotypes | |
|---|---|---|---|
| | | No | % |
| 0–6 | 23 | 33 | 59 |
| 7–12 | 8 | 14 | 64 |
| over 12 | 17 | [illegible]9 | 63 |

Time in months from stopping oral contraceptives to first day of patient s last menstruate period

Carr (4) found in a series of 281 spontaneous abortuses 48% chromosomally abnormal abortuses from women having used OC in comparison to 22% from a control group who had not used OC He also reported a significantly higher frequency of triploidy among the abortuses from women who had used OC A possible explanation of the discrepancy between Carr s material from 1965–1966 and the present one might be that the hormone content of the pills has been reduced

The observation that the mean gestational age of chromosomally abnormal abortuses was longer in the group of women who had used OC than in the other as shown in Table III is difficult to explain especially since no such difference was found for the abortuses with a normal karyotype The difference was significant for the chromosome abnormality 45 X ($P<0.01$) Carr (4) has drawn the attention to a possible after effect of OC on the pituitary gland after stopping OC However such an effect would influence all abortuses in mothers who had used OC and does not explain the observation mentioned above A possible explanation of the findings shown in Table III could be delayed ovulation due to intrafollicular over ripeness It has been shown in animal experiments that induced intrafollicular over ripeness can predispose to chromosomal anomalies in the progeny (12 2) This would however imply that OC do have some kind of effect on the ovary but that this effect has not resulted in a significant increase in the frequency of chromosome aberrations in the present material However a larger series of abortuses might be necessary to detect such a significant increase

## ACKNOWLEDGEMENT

This work was supported by a grant from the Danish Medical Research Foundation

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*Submitted for publication March 18 1974*

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Acta Obstet Gynec Scand 54 265–270 1975

# STUDIES IN NORMAL PREGNANCY

## I *Serum Lipids and Fatty Acid Composition of Serum Phosphoglycerides*

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*Abstract* Elevated s rum lipids in normal pregnancy have been confirmed by the present study In normal pregnancy the relative fatty acid composition of serum lecithin was characteristically high in palmitic acid (16 0) From the pres nt knowledge of lipid metabolism in pregnancy there is no obvious explanation for this finding Furthermore the serum lecithin relative fatty acid composition mirrored a possible dietary influence with a decrease in the essential fatty acid linoleic (18 ?) and arachidonic (?0 4) acids and of the sum of the fatty acid of the linoleic acid series (*n*-6) This expression for a relative deficiency in essential fatty acids might be due to changes in dietary habits during pregnancy e g an increase in particularly refined carbohydrates Reciprocal changes in oleic (18 1) and linoleic (18 2) acids further support this suggestion An expected increase in serum lecithin containing arachidonic acid (?0·4)—due to estrogen influence on liver lecithin synthesis—could not be verified in week 34 of the normal pregnancy

Serum lipids are altered during normal pregnancy Increased serum turbidity in the pregnant woman was discovered more than a hundred years ago (6) Turbidity in serum is preferentially related to increased triglycerides Hyperlipidemia in pregnancy has at least partially been connected with an estrogen influence on lipid metabolism (19 34) since similar changes particularly in serum triglycerides and phosphatides are seen after estrogen administration (13 17 22)

In earlier studies on cholestasis of pregnancy the fatty acid composition of serum lecithin determined by gas liquid chromatography (GLC) was found to be a sensitive indicator for metabolic influences mediated through the lecithin synthesis in the liver (20 21 30)

The aim of the present study was to elucidate the influence of normal pregnancy on serum lipids and particularly on serum phosphoglyceride fatty acid composition

## MATERIALS AND METHODS

*Clinical series*

Twenty women with normal uncomplicated pregnancy randomly selected from the Maternal Welfare Unit were studied The duration of their pregnancies ranged 31–37 weeks (mean 33 6 weeks) and their ages varied from 18 to 35 years (mean 25 5)

Eighteen non pregnant healthy young women with regular periods not using oral contraceptives served as controls Blood specimens were taken on the first or second day of menstrual bleeding Their age varied from 19 to 34 years (mean ?6 ?)

*Blood samples* were drawn in the fasting state in the morning centrifuged at 2 500×g for 10 min and serum immediately recovered frozen and stored at −20°C in glass tubes with Teflon screw caps

*Serum lipids* Cholesterol was determined according to Cramér & Isaksson (11) and triglycerides by the method of Carlsson (10)

*Lipoprotein electrophoresis* was performed on 1% agarose gel in barbital buffer (pH 8 6) ionic strength 0 05 Lipoprotein bands were stained with a mixture of Oil Red O and Fett Rot B (Ciba) and were visually evaluated (15)

*Gas liquid-chromatography (GLC)* Preparation of lipid extract separation of lipids by thin layer-chromatography on Silica gel and isolation of lecithin and phosphoglyceride spots and preparation of fatty acid methyl esters were performed as described by Olegård & Svennerholm (?6)

GLC of methyl esters and conversion from weight per cent to mole per cent were performed as described in an earlier communication (?1)

Except the fatty acid presented in the tables 14 0 15 0 18 3 (*n*-6) 18 3 (*n* 3)+20·1 (*n* 9) 0 3 (*n* 9) 2_ 4 (*n*-6) 22 5 (*n*-6) and 5 (*n* 3) have been identified but not tabulated because their concentrations were generally less than 1% (35)

Table I *Serum cholesterol triglycerides and calculated lecithin in normal pregnancies (n=20 week of gestation mean 33 6 range 30–37 mean age 25.5 years range 18–35 years) and in healthy non pregnant women (n=18 mean age 26.2 years range 19–34 years)*

| Serum lipids (mg/100 ml) | Normal pregnancy (n=20) | | Normal non pregnant (n=18) | | | |
|---|---|---|---|---|---|---|
| | X | S E.M | X | S E.M | Δ | P |
| Cholesterol | 265 | 8 | 206 | 8 | + 59 | |
| Triglycerides | 180 | 13 | 54 | 4 | +126 | |
| Calculated lecithin | 259 | 10 | 198 | 4 | + 61 | |

* =0 001 level

*Quantification of serum lecithin*

Serum lecithin was measured from the fatty acid content (obtained from GLC) using a nomogram The equation $y=1\ 66x+75$ where $y$ is the serum lecithin content as determined by lipid phosphorus (5) and $x$ the amount of lecithin calculated from fatty acid analysis satisfied this experimentally determined relationship (21)

The absolute amount of lecithin (in mg/100 ml) with each particular fatty acid was obtained from the formula Fatty acid (weight per cent)×lecithin (mg/100 ml) The absolute amount of serum lecithin is important to consider when comparing their relative fatty acid composition in state with different serum lecithin level

*Statistical methods*

Conventional methods were used for the calculation of means standard deviations and standard error of means Student s $t$ test was used to study differences between groups Values of $p<0\ 05$ were considered statistically significant (8)

## RESULTS

*Serum lipids* (Table I) Serum cholesterol triglycerides and calculated lecithin were higher ($p<0\ 001$) during pregnancy than in the non pregnant state On lipoprotein electrophoresis the appearance of pre β-lipoproteins (pre β-LP) was evident simultaneously with a more marked α lipoprotein (α LP) (Fig 1)

*Relative fatty acid composition of phosphoglycerides (GPL) as compared with that of lecithin*

Table II *Relative composition of major fatty acids in serum phosphoglycerides (GPL) and lecithin (phosphatidyl choline=PC) in normal pregnancies (n=20 week of gestation mean 33 6 range 30–37 mean age 25.5 years range 18–35 years) and in healthy non pregnant women (n=18 mean age 26 2 range 19–34 years*

Data are given in mole per cent of methyl esters

| | Normal pregnancies | | | | Non pregnant | | | |
|---|---|---|---|---|---|---|---|---|
| | Phospho-glycerides (GPL) | | Lecithin (PC) | | Phospho-glycerides (GPL) | | Lecithin (PC) | |
| | X | S E M | X | S E.M | X | S E M | X | S E M |
| 16 0 | 35 4 | 0 32 | 37 4 | 0 42 | 30 7 | 0 41 | 29 7 | 0 30 |
| 16 1 (n 7) | 1 1 | 0 03 | 1 0 | 0 04 | 0 6 | 0 05 | 0 7 | 0 05 |
| 18 0 | 10 4 | 0 13 | 9 5 | 0 22 | 14 7 | 0 30 | 13 9 | 0 23 |
| 18 1 (n 9) | 12 6 | 0 24 | 12 7 | 0 25 | 11 8 | 0 21 | 11 7 | 0 19 |
| 18 2 (n-6) | 24 2 | 0 48 | 24 9 | 0 44 | 25 8 | 0 88 | 28 5 | 0 69 |
| 20 3 (n-6) | 2 7 | 0 12 | 2 8 | 0 14 | 2 0 | 0 14 | 2 1 | 0 15 |
| 20 4 (n-6) | 6 3 | 0 19 | 5 7 | 0 25 | 7 2 | 0 31 | 6 9 | 0 31 |
| 22 6 (n 3) | 4 8 | 0 19 | 4 0 | 0 24 | 4 4 | 0 27 | 4 2 | 0 26 |
| 18–22 (n 6) | 33 6 | 0 37 | 33 7 | 0 48 | 35 8 | 0 40 | 36 2 | 0 42 |
| 18 2/20 4 | 3 9 | 0 19 | 4 6 | 0 28 | 3 8 | 0 23 | 4 3 | 0 [illegible]6 |
| Fatty acids mg/100 ml | 132 | 5 87 | 111 | 4 17 | 77 9 | 2 76 | 77 4 | 2 59 |

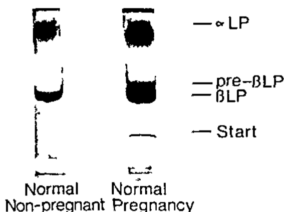



*Fig 1* Examples of lipoprotein electrophoresis in the normal non pregnant and pregnant state (*LP*=lipoprotein) During pregnancy α LP and pre β-LP are increased while β-LP is unchanged

*(PC)* (Table II Table III) When the relative fatty acid composition of GPL (composed of PC cephalin and lysolecithin) was compared with that of PC alone differences were observed

In the *non pregnant state* PC was lower in 16 0 (palmitic acid) ($p<0$ 05) and in 18 0 (stearic acid) ($p<0$ 05) and higher in 18 2 (linoleic acid) ($p<$ 0 05)

Table III *Mean differences (Δ) in relative composition of major fatty acids of serum phosphoglycerides (GPL) and lecithin (phosphatidyl choline=PC) between normal pregnancies (n=20 week of gestation mean 33 6 range 30–37 mean age 25.5 years range 18–35 years) and healthy non pregnant women (n=18 mean age 26 2 years range 19–34 years)*

Data are given in mole per cent of methyl esters

| | Normal pregnancy (*n*=20) GPL-PC | | Non pregnant (*n*=18) GPL PC | |
|---|---|---|---|---|
| | Δ | *P* | Δ | *P* |
| 16 0 | −2 0 | | +1 0 | |
| 16 1 (*n* 7) | +0 1 | – | −0 1 | – |
| 18 0 | +0 9 | | +0 8 | |
| 18 1 (*n* 9) | −0 1 | – | +0 1 | – |
| 18 2 (*n*-6) | −0 7 | – | −2 7 | |
| 20 4 (*n*-6) | −0 1 | – | −0 1 | – |
| ?0 1 (*n* 6) | +0 6 | – | +0 3 | – |
| 22 6 (*n* 3) | +0 8 | | +0 2 | – |
| 18–22 (*n* 6) | −0 1 | – | −0 4 | – |

=0 05 level =0 01 level =0 001 level

Table IV *Mean differences (Δ) in relative composition of major fatty acids of serum phosphoglycerides (GPL) and lecithin (phosphatidyl choline=PC) in normal pregnancies (n=?0 week of gestation mean 33 6 range 30–37 mean age 25.5 years range 18–35 years) and in healthy non pregnant women (n=18 mean age 26.2 years range 19–34 years)*

Data are given in mole per cent of methyl esters

| | Normal pregnancy vs non pregnant GPL-GPL | | Normal pregnancy vs non pregnant PC PC | |
|---|---|---|---|---|
| | Δ | *P* | Δ | *P* |
| 16 0 | +4 7 | | +7 7 | |
| 16 1 (*n* 7) | +0 6 | | +0 3 | |
| 18 0 | −4 3 | | −4 4 | |
| 18 1 (*n* 9) | +0 8 | | +1 0 | |
| 18 2 (*n*-6) | −1 6 | – | −3 6 | |
| 20·3 (*n*-6) | +0 7 | | +0 7 | |
| 20 4 (*n*-6) | −0 9 | | −1 2 | |
| 22 6 (*n* 3) | +0 4 | – | −0 2 | – |
| 18–22 (*n*-6) | −2 2 | | −? 5 | |

=0 05 level *=0 01 level =0 001 level

During *normal pregnancy* PC was characteristically high ($p<0$ 001) in 16 0 (palmitic acid) As in the non pregnant state 18 0 (stearic acid) was lower ($p<0$ 01) in PC than in GPL

*Relative fatty acid composition in GPL and PC in normal pregnancy as compared with that in the non pregnant state* (Table IV) In pregnancy the relative fatty acid composition was characterized by

1 a relatively higher content of 16 0 (palmitic acid) in GPL ($p<0$ 001) as well as in PC ($p<$ 0 001)
2 a lower content of 18 0 (stearic acid) in GPL ($p<0$ 001) as well as in PC ($p<0$ 001)
3 a higher content of 18 1 (oleic acid) preferentially in PC ($p<0$ 01) but also in GPL ($p<$ 0 05)
4 a lower content of 20 4 (arachidonic acid) preferentially in PC ($p<0$ 01) but also in GPL ($p<$ 0 05)
5 a lower content of 18 2 (linoleic acid) in PC ($p<0$ 001) and
6 a lower content of all fatty acids of the linoleic acid series (sum of *n*-6) both in GPL ($p<0$ 001) and in PC ($p<0$ 01)

*Absolute content of PC fatty acids in normal pregnancy as compared to that in the non pregnant*

Table V *Content of total serum lecithin and serum lecithin (PC) with particular fatty acids in normal pregnancies (n=20 weeks of gestation mean 33 6 range 30–37 mean age 25 5 range 18–35) and in healthy non pregnant women (n=18 mean age 26 2 range 19–34)*

Data are given in mg/100 ml

| Fatty acid in lecithin | Normal pregnancy Lecithin (mg/100 ml) | Normal non pregnant Lecithin (mg/100 ml) | Δ | Normal pregnancy vs Normal non pregnant *P* |
|---|---|---|---|---|
| 16 0 | 89 8 | 54 6 | +35 2 | |
| 18 0 | 25 3 | 28 2 | − 2 9 | |
| 18 1 (*n* 9) | 33 2 | 23 5 | + 9 7 | |
| 18 2 (*n* 6) | 65 2 | 57 1 | + 8 1 | |
| 20 4 (*n*-6) | 16 2 | 15 0 | + 1 2 | – |
| 18–22 (*n*-6) | 90 9 | 74 9 | +16 0 | * |
| Total serum lecithin (mg/100 ml) | 259 | 198 | +61 | * |

*=0 001 level

*state* (Table V) Calculated on absolute amounts of lecithin (in mg/100 ml) i e concentration of lecithin with a particular fatty acid (taking into account serum lecithin increase in pregnancy) the increase in 16 0 (palmitic acid) and 18 1 (oleic acid) was even more marked and the decrease in 18 0 (stearic acid) ($p<0\ 001$) remained

The decrease in relative amounts of 18 2 (linoleic acid) 20 4 (arachidonic acid) and sum of *n* 6 respectively turned into an increase in absolute amounts of lecithin with these particular fatty acids ($p<0\ 001$ N S and $p<0\ 01$ respectively)

## DISCUSSION

The present data show in agreement with earlier studies (3 33 34) that serum lipids are elevated in normal pregnancy Serum triglycerides were most markedly increased (by 230%) while serum cholesterol and lecithin were elevated by 22 and 23% respectively On serum lipoprotein electrophoresis a concomitant increase in pre $\beta$ and $\alpha$ lipoproteins (LP) was experienced These latter findings are in agreement with our earlier data (12) Utilizing preparative ultracentrifugation we found in normal pregnancy VLDL (pre $\beta$ LP) with a high content of cholesterol and HDL ($\alpha$ LP) high in triglycerides In normal pregnancy Aurell & Cramér (3) found a high triglyceride content in HDL isolated by column chromatography

Usually in earlier studies the relative fatty acid composition has been given for serum phosphoglycerides i e lecithin lysolecithin and cephalin rather than for lecithin alone Since lecithin composes 85–90% of phosphoglycerides (GPL) in serum the fatty acid composition of GPL usually mirrors that of lecithin (PC) The influence by pregnancy on the relative content of palmitic acid (16 0) was parallel in phosphoglycerides (GPL) and lecithin (PC) In the present study thus the fatty acid composition of lecithin (PC) was chosen and considered an indicator for metabolic influences on liver lecithin synthesis during pregnancy

The relative fatty acid composition in serum lecithin was in normal pregnancy—as compared to the non pregnant state—characterized by high palmitic acid (16 0) simultaneously with a low stearic acid (18 0) content Furthermore linoleic acid (18 2) and arachidonic acid (20 4) and sum of fatty acid of linoleic acid series (*n* 6) were low simultaneously with high mono enoic fatty acids 16 1 (*n* 7) and 18 1 (*n* 9) (oleic acid)

As serum lecithin was markedly increased by pregnancy the influences on serum lecithin fatty acid content will be somewhat different when calculated on absolute amounts Thus pregnancy was characterized by an absolute increase in lecithin containing palmitic acid (16 0) and linoleic acid (18 2) and absolute decrease in stearic acid (18 0) and unchanged amounts of lecithin containing arachidonic acid (20 4)

The reciprocal changes by pregnancy in the relative content of palmitic (16 0) and stearic (18 0)

acids in serum lecithin had no obvious explanation from present knowledge in lipid metabolism Palmitic and stearic acid are those fatty acids usually found in 1 position in human serum lecithin (4 14 23) It is also known that these fatty acids are representative for one lecithin synthesis pathway each A high relative content of palmitic acid in serum lecithin would then suggest an enhancement of pathway I (Kennedy pathway) in the liver (27 28)

A low relative content of linoleic (18 2) and arachidonic (20 4) acids and of fatty acids of linoleic acid series (*n*-6) would suggest a dietary influence i e a relative deficiency of essential fatty acids during pregnancy This pattern in the relative fatty acid composition concomitant with a relative increase in oleic acid (18 1) and palmitoleic acid (16 1) is found in linoleic acid deficiency (1) as well as after sucrose feeding (2) In pregnancy a high sucrose intake has been suggested as an explanation for increased serum triglycerides as well as increased body fat (9 18) Hyperphagia and saccaromania have been linked with increased plasma insulin Hyperinsulinemia has been verified in normal pregnancy (12 32) Among several possible causes for hyperinsulinemia during pregnancy the influence by human placental lactogen (HPL) on carbohydrate metabolism is the most intriguing (29)

The influence of estrogen on liver lecithin synthesis has been shown in in vitro experiments in man (25) and in the animal (4) and in vivo in the animal (7 24) Reciprocal changes in linoleic (18 2) and arachidonic (20 4) acids in HDL lecithin in young females during the menstrual cycle has been taken as evidence that similar estrogen effects also occur in vivo (16) In the present study in normal pregnancy during gestational week 34 an expected enhancement by estrogen on the liver lecithin synthesis pathway II (Greenberg pathway) could not be confirmed at least not from the serum lecithin relative fatty acid composition No increase in serum lecithin with stearic acid (18 0) in 1 position and arachidonic acid (20 4) in 2 position was encountered On the contrary the major synthesis pathway the Kennedy pathway appears also to be dominant in pregnancy The lack of estrogenic influence on liver lecithin synthesis might be due to the particularly high release of gestagenic (anti estrogenic) hormones in gestational week 34 (31)

## ACKNOWLEDGEMENTS

Mrs Margareta Medberg is acknowledged for technical assistance and Miss Annika Höfde for preparing the manuscript

This project was supported by grants from the Swedish Medical Research Council (B74-19X 2100-19A) and the Swedish Oleo-Margarine Association for Nutritional Research

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*Submitted for publication March 27 1974*

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Acta Obstet Gynec Scand 54 271–280 1975

# FREQUENCY AND MANAGEMENT OF UROLOGICAL AND SOME OTHER COMPLICATIONS FOLLOWING RADICAL SURGERY FOR CARCINOMA OF THE CERVIX UTERI STAGES I AND II

*A Five year Analysis of 202 Cases*

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*Abstract* A series of 202 patients with stage I and II carcinoma of the cervix uteri is presented. Radical hysterectomy and pelvic lymph node dissection was performed in all cases and most patients received radiotherapy in addition. The condition at 5 year follow-up of all patients is reported and the facts associated with operative complications are analysed. Special attention was paid to the urological condition by performing urographies as a routine study both pre and postoperatively. There was a strong correlation between the pathological postoperative urologic finding in regard to poor prognosis and permanent complications. Urinary tract infections occurred frequently and were difficult to handle. The operations performed for urological complications are listed. It is concluded that the treatment of carcinoma of the cervix uteri should be concentrated on clinics with a well trained staff. At every stage of treatment intensive teamwork is most important. The rate of complications can be reduced by intensifying studies especially in the postoperative period. New diagnostic procedures and prophylactic measures should be tried and introduced into routine practice if proved valuable. It is important that major surgery such as radical hysterectomy be performed in a teaching hospital for gynecologists.

In clinics with facilities for both radiological and surgical treatment there has been a tendency to combine these forms of therapy when treating cervical carcinoma. In the 1950s the results obtained by combined treatment of stage I and stage II carcinoma of the cervix uteri in the First and Second Department of Obstetrics and Gynecology of Helsinki University Central Hospital were encouraging (26).

From the teaching point of view it is important that the operations performed at university clinics are major operations such as radical hysterectomy and this is no doubt one reason why radical surgery (radical hysterectomy and bilateral lymph node dissection) has been on the increase at our hospital since the 1960s. For a successful outcome it was fortunate that the treatment was planned and carried out by a team consisting of a gynecologist a radiologist and physicist. This enabled closer attention to be paid to all the different aspects. One important fact which in the early 1960s began to attract more attention in the literature was the great number of urological complications following the treatment of cervical carcinoma (9 12).

By 1963 considerable experience had been gained of both radiotherapy and radical surgery of carcinoma of the cervix uteri in our hospital. It was decided to follow up more closely both early and late complications by performing among other things postoperative urographical examinations as a routine procedure. From 1963 to 1967 in most cases the same team was responsible for the treatment of patients with cervical carcinoma in the First and Second Department of Obstetrics and Gynecology and attempts were made to follow set treatment principles. About one half of the patients with stage I and II carcinoma of the cervix received radiotherapy only and the other half was treated with radical surgery which was generally combined with postoperative radiotherapy. The latter patients are analysed here. The present series will represent a suitable control group for future studies.

The following aspects are considered in this study

1 Treatment results after a 5 year follow up in all cases

Table I *Patients according to clinical stage*

| Stage | No of patients |
|---|---|
| I a | 16 |
| I b | 141 |
| II a | 34 |
| II b | 11 |
| Total no of patients | 202 |

2 Complications occurring in the various stages of treatment in both the early and late postoperative period

3 The treatment of these complications and its results

4 By a critical evaluation of the material attempts are made to detect the factors contributing to these complications in order to reduce the frequency of complications without affecting future treatment results

## MATERIAL

The present series consists of 202 patients with cervical carcinoma stage I and II whose treatment included radical surgery The patients were admitted to the First and Second Department of Obstetrics and Gynecology of Helsinki University Central Hospital during the years 1963–67 The present series does not include recidive cases nor those in which it was impossible to perform radical hysterectomy with bilateral lymph node dissection Such cases were excluded from the series because matters associated with radical surgery were to be studied

The preoperative diagnostic specimen for histological study was taken at colposcopy fractionated curettage or combined cone biopsy and amputation of the cervix Histology was in all cases indisputable and the specimens were examined by the same experienced pathologist

Table II *Age distribution of patients*

| Age | No of patients |
|---|---|
| 25–29 | 6 |
| 30–34 | 15 |
| 35–39 | 24 |
| 40–44 | 46 |
| 45–49 | 51 |
| 50–54 | 36 |
| 55–59 | 14 |
| 60–64 | 6 |
| 65–69 | 4 |
| Total no of patients | 202 |

Table III *Hemoglobin values on admission before treatment*

| Hemoglobin (g%) | No of patients |
|---|---|
| 6 0– 9 5 | 7 |
| 9 6–10 5 | 7 |
| 10 6–11 5 | 21 |
| 11 6–12 5 | 70 |
| 12 6–13.5 | 74 |
| ≥13 6 | 23 |
| Total no of patients | 202 |

There were 187 cases of squamous and 15 cases of adenocarcinoma Clinical staging was done according to FIGO prior to treatment An internist examined the patients before operation

The distribution of the patients by disease stage is seen in Table I Table II showing their age distribution The average age for the entire series was 45 2 years the youngest patient being 25 and the oldest 68 Of the patients 17 were nulliparous and 4 pregnant Apart from clinical and gynecological examinations blood and urine were also examined before treatment On admission 25 patients had a significant bacteriuria The hemoglobin values on admission are seen in Table III Only 7 patients had severe anemia

The majority of the patients came to follow-up examinations in our hospital Although some of them had emigrated information on the condition at least 5 years after operation was obtained for all the 202 patients Since death certificates do not always contain reliable information as to the cause of death hospital records and autopsy protocols were obtained for patients who died in other hospitals

## METHODS

The team consisting of a gynecologist a radiologist and a physicist planned the treatment and was responsible for it at its different stages In our clinics the combined treatment includes radical surgery which was performed on those operable patients who were willing to undergo it

Table IV *Patients according to stage and outcome at fifth postoperative year*

| Stage | No of patients | Surviving patients at fifth year | |
|---|---|---|---|
| | | *n* | % |
| I a | 16 | 16 | 100 |
| I b | 141 | 123 | 87 2 |
| II a | 34 | 21 | 61 8 |
| II b | 11 | 6 | 54 5 |
| Total | 202 | 166 | 82 2 |

Table V *Major operative and immediate post operative complications (within 30 days after the operation)*

| Complication | No of patients n | % |
|---|---|---|
| Lesion of right ureter | 1 | 0 5 |
| Lesion of left ureter | 2 | 1 0 |
| Lesion of bladder | 1 | 0.5 |
| Nerve injury | 1 | 0 5 |
| Hematoma or rupture of wound | 7 | 3 5 |
| Pulmonary embolus | 1 (death) | 0 5 |
| Abscess of pelvis | 1 | 0 5 |

*The treatment principles were as follows* the patients received one preoperative radium application according to the modified Stockholm method (?7) The bladder and rectum doses were measured and radiophysical factors observed The surgery radical hysterectomy with bi'a eral lymph node dissection was carried out approximately 2 weeks after the radiotherapy The operation team was headed by a senior surgeon A modified type of Wert heim s operation as described by Werner & Sederl (?9) was performed It should be borne in mind however that surgeons differ as far as the radicality of an operation is concerned The procedure for the treatment of the surgi cal specimen was always the same and the site of every excised node was recorded prior to the histological ex amination

When carcinomatous growth was detected in the histological examination the patients were given external postoperative radiotherapy which was started 4 weeks after the operation provided that recovery had been un eventful The treatment was given using Muller s Rontgen apparatus and it was administered in the form of pendulum convergence therapy Lead shields were used to protect the bones in the pelvis and each side was treated separate ly The average dose to the parametrium was 4000 R A great deal of experience had been gained from this treat ment for the equipment had been in use since 1953 Ex ternal therapy was not given to 17 patients (8 4%) The planned postoperative urographical examination was car ried out whenever possible

The patients came regularly to the out patient depart ment for follow up examination performed at 2 to 6-month intervals during the first 5 years If needed the patients came earlier than scheduled In addition to the routine investigations complementary special ex aminations such as cystoscopy sigmoidoscopy and radiodiagnostic studies were performed in connection with the follow up examinations when necessary Special attention was paid to the condition of the urinary tract This was done in order to detect treatment complications and recidive cases as early as possible

## RESULTS

Since the purpose of the study was to get a picture of the complications occurring in the early and late postoperative period special attention was paid to thorough follow up studies Even though a selected series was involved it was considered appropriate to check the general treatment results Table IV shows the 5 year treatment results A total of 36 patients died during the follow up period Of these patients 5 died of other causes than cervical carcinoma presumably without recurrence In cluded in the latter is one case of postoperative pulmonary embolus The cause of death was fully proved in all cases except two

Apart from damages found at operation compli cations occurring 30 days after operation have also been regarded as immediate surgical complications These are shown in Table V The operative death rate was 0 5% one patient dying of pulmonary embolus on the 6th postoperative day A lesion of the bladder and one of the ureter was detected and repaired at operation Another lesion of the ureter was repaired 3 weeks after the operation

Table VI shows the fistulae which occurred in the present series and their treatment Almost ev ery patient with fistula had a recidivation in the minor pelvis and died of cervical carcinoma One patient with both vesicovaginal and rectovaginal fistula was treated three times for vaginal recur rence This patient has survived with colostomy

Table VI *Fistulae and the result of their treatment*

| *Fistula* | Treatment | Recurrence | Present condition |
|---|---|---|---|
| Uretero-vaginal | Ileo-ureteroplastica | Yes | Death (16 mo ) |
| Vesico-vaginal | Laparotomy explorat | Yes | Death (?0 mo ) |
| Colo-vaginal | Conservative | Yes | Death (34 mo ) |
| *Vesico-rectal* | *Colostomy* | Yes | *Death (18 mo )* |
| Vesico-rectal | Conervative | Yes | Death (31 mo ) |
| Recto-vaginal | Colostomy | No | Alive with colostomy |
| Vesico- and recto-vaginal | Colostomy | Yes | Alive with colostomy |

Table VII *Operations for urological complications other than fistulae occurrence treatment and results*

| Operative procedure | Time of treatment after Wertheim s operation | Primary result | Present condition |
|---|---|---|---|
| Suturatio vuln vesicae urinariae | At operation | Good | Alive |
| Implantatio ureteris dx in vesic urinariae | At operation | Good | Dead |
| Operatio ileo-uretero-plastica l sin | 3 weeks | Good | Dead |
| Deliberatio ureteris sin et evacuatio cystae pelvis | 3 months | Good | Alive |
| Ureterolysis l sin | 3 months | Good | Dead |
| Ureterocystostomia l sin | 4 months | Fair | Dead |
| Ureteroplastia l sin | 8 months | Good | Alive |
| Ureteroplastia l dx | 9 months | Good | Alive |
| (*a*) Ureterolysis l sin | 18 months | Poor | |
| (*b*) Ureteroplastia l sin | 20 months | Good | Alive |
| Implantatio ureteris in sigmae a m Coffey | 19 months | Fair | Dead |
| Ureteroplastia l sin | 35 months | Good | Alive |

In order to clarify current urological condition control urography had to be performed on a large number of patients Lesions in the ureters were always repaired if conservative treatment would have led to permanent damage to the kidney In spite of this the majority of the permanent complications were urological As seen from Table VII complications requiring surgery were usually revealed during the first and second postoperative year

Table VIII *Metastatic spread to lymph nodes and 5 year survival*

| Metastatic spread to nodes | Patients Alive | Dead | Total |
|---|---|---|---|
| Lymph nodes l dx | 3 | 4 | 7 |
| Lymph nodes l sin | 4 | 6 | 10 |
| Lymph nodes l a | 3 | 6 | 9 |
| Total number | 10 | 16 | 26 |

Table IX *Palpation at operation in 26 cases of metastatic lymph node involvement (only clearly suspected cases are noted)*

| Histologically verified metastatic node involvement according to side | Palpation at operation Suspected | Not suspected | Total |
|---|---|---|---|
| Metastases in nodes l dx | 6 | 1 | 7 |
| Metastases in nodes l sin | 0 | 10 | 10 |
| Metastases in nodes l a | 3 | 6 | 9 |
| Total number of patients | 9 | 17 | 6 |

A lymph node metastasis finding is acknowledged to be the most important factor affecting the prognosis of patients with cervical carcinoma (4 13 ?1) The lymph node metastases are presented in Table VIII Prognosis is poorest if metastases are found in several nodes bilaterally Table IX shows how palpation at operation correlates with the histological finding of node metastasis Only such findings recorded by the senior surgeon in the surgical record as enlarged nodes and suspect metastases have been included Only one third of the cases of node metastases were suspect on this basis This kind of suspect finding occurred in only one of the 20 patients who died during the follow up period the histological examination of these patients operative specimen did not reveal node metastases *Table X shows a* comparison of on one hand the exitus of node negative and node positive patients and on the other hand the time elapsed from the operation to exitus *Of the node negative* patients 156 out of 176 (88 6%) and of the node positive patients 10 *out of* 26 (38 5%) were alive more than 5 years after the operation Still better results have been presented on node positive patients in the literature (3 6) Of the 15 patients with adenocarcinoma 5 have died Two of these patients both stage IIA had lymph node *metastases*

Of all the patients 4 were pregnant and had squamous carcinoma and all of them received post

*Fig 1* (*a*) Normal urography finding prior to operation Dec 16 1966 At control urography on Jan 0 1967 slight stasis was seen on the left side Control examination Feb 20 showed progression and in addition deformity of the bladder (*arrows*) (*b*) (*c*) Condition on Mar 17 twelve days after an attempt to free the left ureter Later on evacuation of a pelvic cyst had to be performed The situation was normalized Last control Mar 27 1969 (*d*)

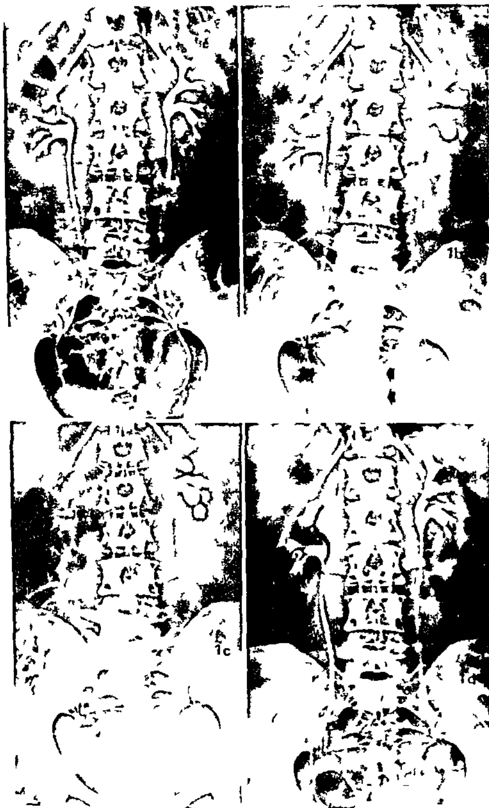

1b
1c
1d

Table X *Survival times in patients with and without lymph node involvement*

| Time of death | Lymph node Positive | Negative | Total |
|---|---|---|---|
| Within 1 year | 4 | 4 | 8 |
| Within 2 years | 5 | 5 | 10 |
| Within 3 years | 2 | 6 | 8 |
| Within 4 years | 3 | 2 | 5 |
| Within 5 years | 2 | 2 | 4 |
| After 5 years | 0 | 1 | 1 |
| Total number | 16 | 20 | 36 |

operative radiotherapy One of these 4 died of *cervical carcinoma She had extensive lymph node* metastases bilaterally and pelvic exenteration was performed 6 months after the radical hysterectomy The patient died 5 months after the exenteration and at autopsy very extensive metastasizing was revealed Pregnancy in connection with carcinoma of the cervix presents special problems that have to be dealt with individually as pointed out by Vara (28)

On *almost every patient urography* was performed prior to surgery in the Radiodiagnostic Department of our hospital Control urography was scheduled for approximately 2 months after the operation In order to evaluate the postoperative urological condition 168 patients with normal preoperative urography and a control examination 3–12 weeks after the operation were selected The following changes revealed in the urography were typical of postoperative uropathy the nephrogram phase was accentuated and the pyelogram phase was *delayed Additionally hydronephrosis* and hydro-ureter of different degrees were revealed The changes were often reversible Careful follow

Table XI *Patients with normal preoperative intravenous urography and abnormal postoperative urography within 3 months after the operation*

| Abnormal finding | No of cases |
|---|---|
| Hydronephrosis 1 dx | 23 |
| Hydronephrosis 1 sin | 24 |
| Hydronephrosis 1 a | 11 |
| Non functioning kidney 1 sin | 1 |
| *Hydronephrosis* 1 sin and non functioning kidney 1 dx | 2 |
| Total no | 61 |

Table XII *Urological condition at 5 year follow up in 61 patients with abnormal postoperative intravenous urography*

| | |
|---|---|
| Alive normal urography on control examination | 33 |
| Alive permanent abnormal urography | 13 |
| Exitus dead normal urography before death | 4 |
| Exitus abnormal finding on control urography | 6 |
| Exitus control of postoperative urography not performed | 5 |
| Total | 61 |

up after such a pathological finding is necessary in order to avoid permanent damage to the kidney (23)

*Fig 1 (a–d)* shows a series of *urographies* performed on a patient who had undergone repair surgery The final outcome was good Fig 2 (*a–d*) is an example of conservative treatment with spontaneous recovery The urographical finding was postoperatively pathological in 61 out of 168 patients (36 3 %) This high figure is at least partly due to the fact that even slight changes could be *noted when comparing preoperative and postoperative* examinations both performed using the same technique Table XI presents the pathological postoperative findings of urography The reversibility of these changes and the condition of the patients at later follow up examinations are shown in Table XII Thirteen of the patients with an abnormal postoperative urography had permanent changes of differing degrees of severity

Groups of patients with normal and abnormal postoperative urographical findings are compared *in Table XIII from which it can be seen that* the postoperative finding is of obvious importance both for the prognosis and the frequency of permanent complications The significance of urinary tract infection as a contributing factor to complications has been emphasized in several studies (11 24) In the present series 25 patients had a significant bacteriuria on admission In spite of prophylactic treatment 39 patients had postoperative urinary

*Fig 2* (*a*) An example of spontaneous recovery without operative treatment *Normal finding prior* to operation June 7 1967 (*b*) A 3 hour film showing strong stasis June 21 1967 On August 3 1967 (*c*) there is dilatation of the cavities to the right but left side is normal (*d*) Later control showed improvement and last control Jan 11 1971 shows only slight permanent dilatation of right ureter


2a


2b


2c


2d

Table XIII. *Postoperative finding on intravenous urography and condition of patients at 5 year follow up in 168 cases*

| Condition of patients at follow-up examination | Postoperative urography | |
|---|---|---|
| | Normal | Abnormal |
| Alive and well | 84 | 33 |
| Alive with complications | 5 | 12 |
| Alive with recurrent disease | 2 | 1 |
| Dead | 16 | 15 |
| Number of patients | 107 | 61 |

tract infection (UTI). Of these patients, 23 had recidive infections, which were difficult to cure. Even at a later stage UTI often occurred, even in patients with no additional complaint. In the present series a clear connection was also seen between the urological finding and the UTI. Only 16 out of the 107 patients (14.9%) with normal postoperative urography had a postoperative UTI, whereas 23 out of the 61 patients (37.7%) with a pathological urographical finding had UTI.

Table XIV presents a general review of the condition of the patients on the basis of the last follow up examination, at least 5 years after the operation.

## DISCUSSION

Many forms of treatment including more or less radical surgery are applied in the treatment of cervical carcinoma (1, 5, 8, 14, 16, 22). The present series gives a picture of some of the problems connected with surgical treatment of cervical carcinoma. Certainly some complications are always to be expected in connection with radical surgery. The only unacceptable way of trying to reduce their number is *not* to perform a sufficiently radical operation.

Of the positive aspects established in the present series the foremost is a low operative mortality, which was obviously the result of a well managed thromboembolic prophylaxis (25) and the preoperative examination by an internist.

One of the foremost negative aspects is that permanent severe complications due to surgery could not always be avoided. The frequency of these complications, however, is not of an alarming magnitude in comparison with figures in previous reports.

Persistent and recidive UTI presented a great problem. Gitsch et al. (7) were able to reduce the frequency of complications considerably by making postoperative prophylaxis more effective.

Surgery gives a more accurate picture of the extent of the disease than clinical staging. The degree of severity often proves to be considerably greater than can be concluded from clinical examination (3, 17). It is well known that the prognosis of patients with node metastasis is still deplorably poor in comparison with the prognosis of cases with no metastasizing. For cases of degree Ia, Kolstad (15) has suggested less radical surgery and this also seems to find support in the present series.

The prognostic significance of a postoperative urographical finding is surprisingly great in regard to mortality and permanent complications. This was shown by selecting for analysis those 168 patients in whom even slight changes in postoperative urography could be verified. It has also been established in large series that a pathological finding revealed in urography prior to treatment is highly significant for the final result (6). Thus, postoperative control urography can be considered essential.

Our experiences during recent years in our clinics are in conformity with those of Lundgren et al. (19) who have suggested that a normal renographical finding excludes pathological condition and recommend control urography for cases in which the renographical finding is not normal. Renography enables an earlier detection of changes postoperatively and, additionally, a more effective follow up.

The first 3 postoperative years in particular are especially important with regard to recurrence. If urography is postoperatively verified as normal but later is found to be pathological, such a finding is strongly indicative of recurrence. On comparing the

Table XIV. *Present condition of all patients. Last follow up between 5 and 10 years after operation*

| Condition of patients | No. of patients |
|---|---|
| 1 Alive without objective complications | 144 |
| 2 Alive with minor permanent complications | 12 |
| 3 Alive with major permanent complications | 7 |
| 4 Alive with recurrent disease | 3 |
| 5 Postoperative death | 1 |
| 6 Death after postoperative period | 35 |
| Total | 20 |

urological condition of surviving patients to earlier series (2 20) the results can be regarded as acceptable In this respect here is still hope of further reducing the number of complications In order to accomplish this the detection of UTIs and their careful treatment is one of the most important factors (30)

Recently additional examinations have been increasingly used both before and after treatment including lymphography and angiography (18) Though these do not affect clinical staging lymphography for instance is of importance in evaluating the radicality of an operation and angiography in diagnosing recurrence The concentrating of treatment on major treatment centers as suggested by Held (10) among others creates the possibility of providing sufficient experience for the personnel concerned

Based on the analysis of the present series the following conclusions can be drawn

1 An intensive teamwork by the specialists foremost concerned—the gynecologist the internist the physicist and the radiologist—is of the utmost importance at every stage

2 The frequency of complications can be further reduced by more detailed urological examinations especially in the immediate postoperative period Some complications however are always to be expected with this kind of major surgery

3 It is appropriate to try out new diagnostic procedures together with old ones and apply them when they prove valuable Renography is mentioned as an example of this

4 A critical evaluation of the results and complications of treatment should be made from time to time

5 Major treatment centers engaging in relevant research provide the best facilities for effective and successful treatment

## ACKNOWLEDGEMENT

I express my deep gratitude to the Head of Department I Professor Sakari Timonen M D and to the former head of Department II of Obstetrics and Gynecology Professor Paavo Vara M D and the senior doctors at Department I and II of Obstetrics and Gynecology University Central Hospital Helsinki Finland

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*Submitted for publication Nov 26 1974*

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Acta Obstet Gynec Scand 54 281–283 1975

## CASE REPORTS

# HYPERPARATHYROIDISM AND PREGNANCY

*Report of a Case and Review of the Literature*

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*Abstract* A 28 year-old gravida III with a history of two unsuccessful pregnancies was admitted with hyperemesis gravidarum and was found to be suffering from hyperparathyroidism (HPT) She was treated surgically and was later delivered of a wellformed premature girl The literature is reviewed and the histories of 40 females (including the patient of this case report) with a minimum of 93 pregnancies while suffering from HPT shows that HPT during pregnancy is a serious condition for the fetus as well as for the mother There was an increased incidence of spontaneous abortion perinatal death premature birth and neonatal morbidity The mothers suffered from increased episodes of renal calculi and hyperemesis gravidarum The exacerbations nearly always occurred in the first and second trimesters or post partum

Hyperparathyroidism (HPT) is reported to occur in ½% of the adult Danish population somewhat more often in women than in men (26) but it is not diagnosed in nearly as many cases The explanation is that the disease may run a fairly silent course having exacerbations simulating other diseases e g renal calculi duodenal ulcer bone pain constipation and depression Therefore a number of exacerbations usually occur before the true nature of the disease is realized

Latent HPT may be provoked and thus become manifest Provocative factors have been reported to be bed rest operation rough palpation on the neck dehydration and infection (3 32) By means of the present case report and a study of the literature on HPT in connection with pregnancy we are trying to demonstrate that pregnancy too may provoke an exacerbation of HPT

## CASE HISTORY

*Past history* In 1962 the patient aged 19 years had experienced isolated episodes of low back pain and haematuria and had observed passage of calculi She was pregnant for the first time and the expected date of delivery was the middle of September 1965 At the beginning of the pregnancy however she developed severe nausea and vomiting accompanied by weight loss She was admitted to a hospital elsewhere on 9 2 1965 The symptoms subsided on conservative treatment The serum calcium was not determined during this stay in hospital On 23 7 1965 the patient was readmitted to the same hospital as she had now again had nausea and vomiting for 6 days Between the two admissions she had been feeling perfectly well After admission no fetal movements were felt A few days later the patient delivered a stillborn macerated male fetus weight 1 550 g length 39 cm Numerous infarcts were found in the placenta

On the 14 6 1966 the patient again was admitted to the same hospital with hyperemesis gravidarum The size of uterus was found to correspond to 6th–8th week pregnancy It proved impossible to arrest the hyperemesis and uterine curettage was performed Thereafter the patient felt well The serum calcium was not determined

*Present admission* On 23 11 1971 the patient now aged 28 years and in her third pregnancy was admitted to Glostrup Hospital with hyperemesis gravidarum She was in the 9th week of pregnancy During the past 5 weeks she had had nausea and 4–10 vomits daily with a total weight loss of 6 kg The patient was emaciated and dehydrated weighing 37 kg height 163 cm and BP 100/80 Gynaecological examination showed no abnormality The size of uterus corresponded to an 8-week pregnancy Physical examination showed no goitre and no palpable masses on the neck The total serum calcium was elevated 170 mg/l (normal 90–105 mg/l) ultrafiltrable cal ium 99 mg/l (normal 54–64 mg/l) se

rum phosphate 19 mg/l (normal 20–50 mg/l) serum creatinine 11 mg/l creatinine clearance 66 ml/min alkaline phosphatases 6 9 U/ml (normal 3 0–10 0 U/ml) total protein 68 g/l and Hgb 98 g/l A cortisone suppression test was performed with cortisone acetate tablets 50 mg 3 times daily for 10 days Serum calcium remained at an uncharged elevated level throughout the test X rays of the hands revealed a translucency in the distal phalanx of the left little finger Moreover translucencies were found in both medial malleoli Somewhat decreased calcium content in the right humerus Radiography of the chest and dental alveoli showed no abnormalities

After an unsuccessful attempt to find the tumour on 6 12 1971 a new exploration of the neck was made on 29 1? A flat pedunculated greyish red tumour 1×1×½ cm was found on the right of the upper pole of the thyroid gland Histological examination Well-defined adenoma consisting predominantly of fairly large chief cells Minor accumulation of oxyphilic cells and water clear cells Diagnosis Parathyroid adenoma The postoperative course was uneventful

The patient was admitted on 26 5 1972 in the 36th week of gestation as the waters had passed Two days later she was delivered of a girl weighing 1910 g 47 cm in length The baby thrived well and was discharged on 19 6 in a good general condition The serum calcium was normal

At an out patient visit on 3 7 1973 mother as well as infant were in good health and serum calcium was normal

## DISCUSSION

According to the past history this patient has presumably had HPT for 9 years For long periods the course has been fairly silent but there were exacerbations in connection with the pregnancies During all 3 pregnancies the patient developed severe nausea and vomiting A contributary cause of the long course was probably that these symptoms are common complaints also in pregnancies without HPT

Perusing the literature we found reports of 40 pregnant women with HPT (1–2 4–16 18–25 27–31) including the patient of this case report These women went through a minimum of 93 pregnancies while suffering from HPT Of these pregnancies 39 were complicated by purely maternal complaints most often renal calculi skeletal diseases and hyperemesis gravidarum Thus 7 patients (7 15 19 25 30 31) had hyperemesis without this complaint leading to a diagnosis of HPT

It was characteristic of the present case that the symptoms appeared at a very early stage of the pregnancy each time about two weeks after the first missed period Out of the 39 pregnancies reported to have shown purely maternal symptoms we found 22 statements about the time at which the maternal symptoms had set in In 7 pregnancies the symptoms occurred early (2 7 15 19 21 28 31) in 9 cases in the 5th–6th month (4 8 13 18 21 28 31) and in 5 cases post partum (10 16 23 28) In addition to the present patient yet another one (19) has exhibited symptoms in the 3rd trimester The symptoms in the first pregnancy of the present patient returned in the 3rd trimester possibly because of the fall in estriol production caused by intrauterine death of the fetus The symptoms ceased shortly after the pregnancy ended

There is no explanation why the exacerbations in HPT occurred at the times stated but the findings are in keeping with Lehr & Krukowski's observations from experiments on rats (17) These workers found that in the last trimester of pregnancy rats could tolerate an injected dose of parathyroid hormone (PTH) which was fatal in non pregnant rats and in rats in the first and second trimesters

The onset of symptoms at the beginning of pregnancy and their disappearance shortly after the first 2 pregnancies had been terminated in our patient appear to show that pregnancy has an adverse influence upon the course of HPT and may provoke an exacerbation This hypothesis has previously been advanced by Spingarn & Geist (25) and Clerc (7) whose patients had a distinct exacerbation of HPT at the onset of pregnancy Wagner et al (28) analysing a series of 23 women having a minimum of 34 pregnancies in the course of their HPT compared the findings with those in 138 patients making up a consecutive series of HPT They found the serum calcium level in the pregnant patients to be on average higher than among the non pregnant patients They also observed that the mean period from the initial sign of HPT until it was diagnosed was half as long in pregnant women with HPT as in the non pregnant patients The comparison showed moreover that among pregnant patients there was a somewhat higher incidence of skeletal changes than in non pregnant patients

Also for the fetus HPT is a serious complication Among the 93 cases were 21 instances of spontaneous abortion or perinatal death In a further 16 cases there occurred premature deliveries and neonatal morbidity especially neonatal tetany

Thus HPT is a serious complication of pregnancy—just as pregnancy is a serious complication of HPT The diagnosis is made in pregnant patients on the basis of the same criteria as in non pregnant persons i e primarily by demonstrating an elevated total serum calcium or (better) by demonstrating elevated ultrafiltrable calcium or ionized calcium For the purpose of differentiating between hypercalcaemia due to HPT and hypercalcaemia due to other causes a cortisone suppression test was performed in the present case It did not give rise to side effects in the mother or infant

In 1947 Petit & Clark (21) were the first to remove a parathyroid adenoma successfully in a pregnant patient Since then this operation has been carried out in 5 cases (8 12 16 18 22) every time with a good result Our patient also stood the operation well


## ACKNOWLEDGEMENT

Thanks to Dr Herluf Jensen Medical Department B Glostrup Hospital for great help with this case report


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*Submitted for publication Oct 23 1973*

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The advantages of this culdoscopic operation are

1 Therapeutic surgical treatment saving a laparotomy

2 No need for general anesthesia (and anesthetist)

3 Short stay in hospital

4 No abdominal scar which is of great importance to the unmarried patient

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*Submitted for publication March 2? 1974*



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Acta Obstet Gynecol Scand 54 287–292 1975

# DIFFUSE VAGINAL ADENOSIS

*Three Cases Combined with Imperforate Hymen and Haematocolpos*

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*Abstract* Diffuse vaginal adenosis is a rare disease presenting severe symptoms and difficult to treat It has not previously been described in the Scandinavian literature Three typical cases are reported two in children of 14 years and one in a woman of 20 years who were all treated for imperforate hymen and haematocolpos None of the patients had been subjected to oestrogen treatment in utero The colposcopic findings are very characteristic and are described in relation to the histological examination The pathogenesis treatment and risk of malignant transformation are discussed

Reports in recent years from the USA of the sudden appearance of nearly one hundred cases of vaginal and cervical adenocarcinoma in young women below the age of 30 years have created increased interest in the occurrence of vaginal adenosis Thus in 1972 Herbst et al (6) stated that they had demonstrated the presence of adenosis in parts of the vagina neighbouring areas of vaginal adenocarcinoma in 24 of 26 cases The majority of mothers to these women had been treated during pregnancy with synthetic non steroid oestrogen hormones

It is not known how often diffuse adenosis occurs together with imperforate hymen and haematocolpos Ottolenghi Preti (7) has made no mention of the occurrence of vaginal adenosis in a survey of reports published to date on imperforate hymen and haematocolpos We have only been able to find in the literature the case reported by Ruffolo in 1971 (9) of vaginal adenocarcinoma arising from vaginal adenosis however this patient had been treated for imperforate hymen and haematocolpos some 20 years earlier By 1968 sixty nine cases of benign adenosis had been described (10) the diffuse form of the disease comprised at the most 25% of these cases

There is the possibility that vaginal adenosis frequently occurs in connection with imperforate hymen and haematocolpos and that the condition is overlooked as the symptoms can be transitory We therefore consider it of interest to report the following three cases

## CASE STUDY

*Case I*

The patient has been observed for 5 years She was admitted acute at the age of 13 years to the surgical department owing to abdominal pain and the diagnosis of imperforate hymen and haematocolpos was made On puncture of the hymen 300 ml of slimy menstruational blood were removed and hymenal plastic was performed The postoperative course was uncomplicated

The patient complained during the following 2 years of increasing slimy sticky vaginal discharge This required the wearing of a sanitary towel and several changes a day were necessary Gynaecological examination was repeatedly carried out and a slimy vaginal discharge was noted The portio was red and granulated similar areas were found within the vagina particularly along the lateral walls but also on the anterior and posterior walls

Two years after the hymenectomy the first colposcopic examination was performed and the diagnosis Vaginal adenosis was made on colposcopic directed biopsies Microscopical examination of the vaginal discharge showed Döderlein s bacilla and many leucocytes No trichomo-

nas cluecells or hyphomycetes similarly no growth was found on culture for both bacteria and fungus Urography and cystoscopy were normal The hormonal status of the patient as evaluated by pituitary gonadotropin (4 MU/24 hours) and oestrogen excretion (32 micrograms/24 hours) was normal as was the menstruational cycle

Oral contraceptive treatment (Enavid 5 mg®) was given for the following 9 months as a contraceptive measure and in order to observe the effect of this treatment on the development of the adenosis

In the mean time the patient commenced sexual life Bleeding then often occurred during coitus and she complained of vaginal dyspareunia

The anti-ovulation treatment was discontinued after 10 months as the patient wished to become pregnant She conceived 8 months after discontinuation of the drug The pregnancy ran an uncomplicated course and she gave birth to a normal child at term

The character of the discharge remained unchanged both during the medicamental antiovulation treatment and the pregnancy

*Case II*

The patient has been observed for 2 years She was admitted at the age of 20 years to the gynaecological department for primary amenorrhoea and abdominal pain Abdominal examination showed a smooth mass in the midline above the symphysis reaching almost up to the umbilicus Imperforate hymen and haematocolpos were diagnosed No urine retention 1100 ml of slimy menstruational blood were removed on puncture after which hymenectomy was performed The vagina was considerably distended and the uterine cervix wide open The uterus on palpation appeared to be slightly enlarged The salpinges could not be palpated The postoperative course was uncomplicated

The patient complained of slimy sticky discharge at the control examination one month postoperatively Gynaecological examination revealed that the portio was the site of severe eversion and red granulated areas could be seen throughout the whole vagina especially along the lateral walls The uterus was of normal size The diagnosis vaginal adenosis was made by colposcopy and histological examination

Examination of the vaginal discharge showed no trichomonas fungal or haemophilus vaginalis infection but streptococcus faecalis and staphylococcus aureus were found on routine culture

Sixteen months after the hymen plastic operation the patient still complained of continued slimy viscous discharge The cervical ectopy and adenotic areas in the vagina were treated by cryo surgery (−80°C for 30 sec) After which the vaginal discharge diminished and one month after the cryo surgery the granulated areas had decreased in size and were covered by smooth epithelium

*Case III*

The patient has been observed for 2 months She was admitted to the gynaecological department at the age of 14 years owing to acute abdominal pain and abdominal tumour On examination a mass was found situated in the midline extending from the symphysis to the umbilicus

Gynaecological examination showed hypoplasia of the labia minor on the left side Imperforate hymen and haematocolpos were also demonstrated No urine retention Following puncture of the hymen 1200 ml of slimy menstrual blood were removed the vagina was severely distended and the uterine cervix open The uterus was slightly enlarged but the salpinges could not be palpated Hymenectomy was performed and a biopsy taken from several sites in the vagina The postoperative course was uneventful

After the operation the patient complained of a malodorous slimy discharge and examination 5 weeks postoperatively revealed both on the portio and throughout the entire vagina red granulated areas The diagnosis vaginal adenosis was made by colposcopic and histological examination and microscopy of the vaginal discharge showed many rod shaped bacteria together with numerous leucocytes but no trichomonas hyphomycetes or signs of haemophilus vaginalis infection Routine culture produced spread groups of Neissera of the catarrhalis/pharyngis group

*Colposcopy*

The same following characteristic changes were found in all three patients at colposcopic examination

*Cervix* In two of the patients very widespread zones of transformation were seen with ectopy and metaplasia and in the first patient a diffuse villous surface pattern and smooth metaplastic epithelium with severe blood vessel punctation all over the cervix outside of the transformation zone In the last patient the transformation zone was short but well defined Solitary or confluent islets of villouse configuration without signs of metaplasia occurred over the remainder of the cervix

*Vagina* In all three patients there were throughout the vagina as far down as to a few millimeters from the edge of the hymen large areas with pure villouse configuration without signs of metaplasia (Fig 1) as is found in ectopia of the cervix In many areas it was possible to demonstrate a mixture of villose and smooth metaplastic epithelium (Fig 2) In other areas nodular very vascularized projections could be seen Along the lateral walls of the vagina in particular there were ulcerations and nodular surface contours where bleeding could easily be provoked The epithelium between the adenotic areas was found to be smooth with pronounced blood vessels often in the form of coarse punctation (Fig 2)

Hyperplasia of the adenotic areas on the cervix and in the vagina was seen during oral contraception and pregnancy however this was most pronounced in the transformation zone on the cervix where severe metaplasia also occurred At the time at which colposcopy was performed and immediately after removal of the haematocolpos it was impossible to evaluate the condition of the vagina owing to irritation and ulceration During the same examination severe eversion of the cervical canal was present so that it was possible to observe inclined pits and clefts together with a very characteristic network like blood vessel pattern

After cryo treatment the cylindrical epithelium was replaced by smooth metaplastic epithelium within a few weeks

Fig 1 Colpophotography Islets of villouse and smooth metaplastic epithelium ×10

Fig 2 Colpophotography Circumscribed adenotic areas in the anterior wall of the vagina with villi and smooth meta plastic epithelium ×13



*Fig 3* Biopsy from the vagina Grypts covered with cy lindrical and metaplastic multilayered squamous epithe lium ×?00

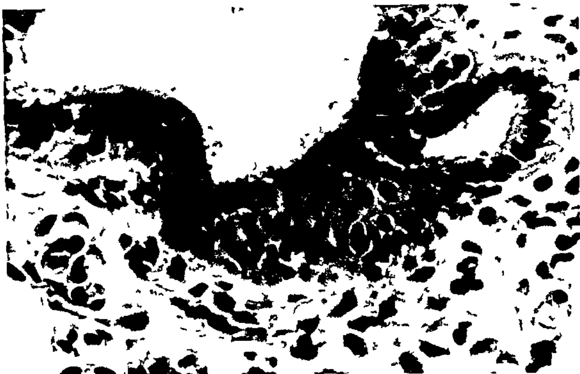


*Fig 4* Biopsy from the vagina. Cubical epithelium and basal layer of metaplastic cells ×350

### *Histology*

The histological changes are of the same character in the three patients The mucosal surface of the vagina varied between areas of squamous epithelium and cylindrical epithelium The squamous epithelium varied in thickness and at places was of obvious metaplastic character (Figs 3 and 4) The cylindrical epithelium differed somewhat in appearance in a number of areas it consisted of a single layer in others it appeared to be pseudostratified In a number of places the areas covered by cylindrical epithelium formed crypts in other areas oval or circular pits covered by cylindrical epithelium were seen in the connective tissue (Fig 3) The latter structures presumably represent tangentially cut crypts On PAS alcian blue staining of the preparations partly at pH 1 and partly pH 2 7 it was possible to observe a slightly varying reaction in the cylindrical epithelium inasmuch as parts reacted as cervical epithelium and others more like tubal epithelium In a few biopsies it was noted that the cylindrical epithelium went over into squamous epithelium and continued as a border of cubic cells on the surface of the squamous epithelium These cubic cells contained alcian blue positive material in the cytoplasm

Widespread adenosis with large areas of epithelial metaplasia was present in the biopsies from the three patients There were no histological signs of malignancy

## DISCUSSION

Two of our patients were only 14 years of age at the time at which adenosis was demonstrated and they are the youngest reported patients who have not been exposed to oestrogen treatment in utero The first patient has been observed for 5 years until the 32nd week of her first pregnancy Neither *oestrogen–gestagen treatment nor the massive* hormonal effect of pregnancy had other influence on the development of the adenosis than that normally found in the transformation zones on the cervix namely some hyperplasia and severe metaplasia in the villose areas (4) A discussion has taken place as to whether the vaginal adenosis is of paramesonephric or endodermal origin from the urogenital sinus It is now generally accepted that the normal squamous epithelium in the vagina is derived from the sinovaginal process which during embryonic life replaces the paramesonephric process (4) presumably up to and including the external orifice of the uterine cervix (3) (5)

Concerning cAMP behaviour during pregnancy so far it is known only that its excretion into the urine towards the end of a normal pregnancy increases by about 50% (12) In toxaemia of late pregnancy the excretion seems to decrease significantly (13) which may be a result of reduced cAMP formation in the cell The cell should develop an alpha receptor effect and the tone of the muscle cell should increase This is in fact what happens in toxaemia uterine contractions and vascular reactivity increase (2) and blood pressure rises

The receptor theory therefore forms a very interesting basis for reviewing many occurrences during pregnancy from a new angle On this basis we can explain the favourable effect of beta sympathomimetic agents as inhibitors of premature delivery since while stimulating the beta receptor of the muscle cell they bring about an increase in cAMP formation (13) The role of estrogens as pregnancy maintaining agents seems understandable since their effect on the uterus is similar to that of cAMP both make it grow larger and activate e g hexokinase and phosphofructokinase (3)

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## BOOKS RECEIVED

*Rheoen ephalographie des Fetus und des Neugeborenen* by Konstantin Cacava VEB Georg Thieme Verlag Leipzig 1974 134 pp 57 illustrations Price Ln 59 – M

A small well illustrated book translated from the Russian language The electrical resistance between two scalp electrodes is method It decreases when the circulation increases and vice versa These measurements are made together with recording of the ECG The technique is used in the USSR for supervision of the fetus during labour

*Gynecologic Surgery Errors Safeguards Salvage* Editor John H Ridley Williams & Wilkins Co Baltimore 1974 317 pp Price $19 50 £8 75

*Problemes pratiques d endocrinologie* edited by J Hazard Masson & Cie Paris 1974 188 pp 50 Figs 25 Tables Price Fr 705

A typically French multi author book which is recom mended to colleagues interested in adolescent gynaecology

*The Teaching of Human Sexual tv in Schools for Health Professionals* World Health Organization Geneva 1974 (Public Health Papers No 57) 47 pages Price Sw fr 5 – French Russian and Spanish editions in preparation

A good survey

*Gynecology A Textbook for Students* by F K Beller K Knorr Ch Launtzen and R M Wynn Springer Study Edition Medicine Springer Verlag New York–Heidelberg–Berlin 1974 385 pages 189 Figs Price Soft cover DM 38 – US $15 50

An excellent small modern text book containing chapters concerning sexual physiology of reproduction fundamen tals of psycho-somatic gynaecology family planning etc

*The New Sex Therapy* by Helen Singer Kaplan Baillière Tindall London 1975 544 pages 20 illustrations Price £7 75

A very instructive text-book recommended to gynaecologists and psychiatrists working in the field of sexuology

*The Placenta Biolog cal and Clinical Aspects* by Moghissi & Hafez Charles C Thomas Publisher Springfield Illinois USA 1974 Price $79 50

The book covers the present knowledge of placenta physiology with interdisciplinary exchange of recent prog ress in basic and cl nical aspects of the placenta and is relating placental struction to it s function Doctors Moghissi and Hafez are well known specialists within the field of human reproduction and their editorial work has been excellently performed The book is recommended to all obstetricians and gynaecologists who want to refresh their knowledge about the human placenta and it s function

*Tumor and Graviditat* by A Verhagen Springer Verlag Berlin 1974 146 pp 17 illustrations 82 tables Price US $77 80 DM 68 –

The book is a compilation from the literature concerning all types of malignancy during pregnancy About 800 refer ences are given The book must be a gold mine for those who intend to publish a paper concerning some type of tumor and pregnancy

*Ovarian Function* Proceedings of the Reinier de Graaf Tercentenary Symposium 8–11 August 1973 Noordwij kerhout The Netherlands Editors T K A B Eskes H L Hautzager and E V van Hall Elsevier Excerpta Medi ca North Holland 1974 285 pp Price US $25 – Dfl 65 00

Consists of the transactions from a symposium which gives a complete up-to-date survey concerning the knowledge within this field The book is recommended to all gynaecologists especially those interested in gynaecological endocrinology

*Advances in Voluntary Stenli ation* Proceedings of the Second International Conference Geneva Switzerland 25 Febr – 1 March 1973 Editor Marilyn E Schima Interna tional Congress Series No 284 Elsevier Excerpta Medica Amsterdam 1974 about 500 pages Price Dfl 67 50 US $74 –

The book gives a good survey of our present knowledge *concerning female and male sterilization and it s conse* quenses in different parts of the world It is recommended to all doctors working in family planning

*Chirurgische Diagnostik* edited by L Leger and M Nagel Springer Verlag Berlin 1974 386 pp 776 illustrations Price DM 48 – US $18 50

An elementary excellent text-book recommended to un dergraduate students

*Publ c Education abo t Cancer 1974* UICC Technical Report Series Volume 11

*Perinatal Pharmacology Problems and Priorities* Editors Joseph Dancis and Joseph C Hwang A Monograph of the National Institute of Child Health and Human Development Raven Press Publishers New York

Concerning cAMP behaviour during pregnancy so far it is known only that its excretion into the urine towards the end of a normal pregnancy increases by about 50% (12) In toxaemia of late pregnancy the excretion seems to decrease significantly (13) which may be a result of reduced cAMP formation in the cell The cell should develop an alpha receptor effect and the tone of the muscle cell should increase This is in fact what happens in toxaemia uterine contractions and vascular reactivity increase (2) and blood pressure rises

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Acta Obstet Gynecol Scand 54 297–301 1975

# OBSTRUCTION OF THE UPPER URINARY TRACT AFTER TREATMENT OF CARCINOMA OF THE UTERINE CERVIX

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*Abstract* The frequency of obstruction of the upper urinary tract after treatment of carcinoma of the uterine cervix was assessed in an investigation of 100 consecutive patients 64 of the women were treated surgically (Wertheim hysterectomy) the remaining 36 with Wertheim hysterectomy combined with radiotherapy (combined treatment) The patients were examined with isotope renography and with i v urography before as well as 14 days 2 months 4–6 months and 1 2 3 4 and 5 years after the operation When necessary these examinations were supplemented by retrograde pyelography intravenous pyelography and selective renal function tests Postoperatively 40 3% of the patients treated with surgery alone showed signs of ureteric obstruction whereas the figure for those treated with the combined treatment was 55% Of the patients 25 developed in the early postoperative course mild ureteric obstruction which disappeared within half a year Such mild obstruction was not regarded as a true complication of the treatment given On the other hand 21 patients developed obstinate ureteric obstruction In 14 of these patients surgical intervention was necessary to save renal function Most of the patients with serious ureteric obstruction had fairly advanced carcinoma (15 of stage 2 and 6 of stage 1) Radiotherapy had been given more often in this group (15 out of 21) than in the rest In 4 of the patients the ureteric obstruction was due to a recurrence of a tumour This means that the true frequency of postoperative ureteric obstruction was 17% In the group given combined treatment urinary stasis persisted longer than in the group treated with surgery alone Renography and urography were done on 687 occasions and the results did not agree in 14%

Several investigations have been published on the urological complications following treatment of carcinoma of the uterine cervix such as urinary tract infection uretero-vaginal and vesico vaginal fistulae as well as disturbances of micturition Thanks to improved surgical and radiotherapeutic methods the frequency of fistulae has decreased in recent years but the incidence of ureteric obstruction after treatment of carcinoma of the uterine cervix still appears to be relatively high (32 16 27 37 24) It is difficult if not impossible to predict the later course of this obstruction it may regress spontaneously or it may progress and lead to hydronephrosis and reduction of renal parenchyma with impairment of renal function Ureteric obstruction therefore requires close observation in order to enable early detection of any progression which may require surgical intervention

Formerly the excretion of urine after treatment of carcinoma of the uterine cervix was checked by intravenous urography alone but in recent years urography is being supplemented or replaced by radioisotope renography (24 28 18)

The aim of this study was to assess the frequency and the later course of ureteric obstruction following treatment of carcinoma of the uterine cervix as judged from i v urography and isotope renography

## MATERIAL AND METHODS

The material consisted of 100 consecutive patients with carcinoma of the uterine cervix stages Ia–2b All of the women were subjected to Wertheim hysterectomy which was supplemented with radiotherapy in 36 of them (Fig 1) We do not as a rule regard operation as indicated if the woman is over 65 years or if the tumour is more advanced than stage b Women in the reproductive age group with a stage Ia–Ib tumour were generally treated with surgery only and when possible both or one of the ovaries were left behind The age distribution of the series is given in Table I When any of the lymph nodes excised proved to be carcino-

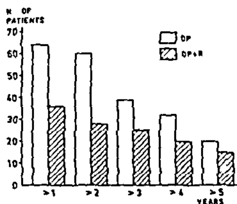



*Fig 1* Number of patients divided into two groups only operated or treated with a combination of surgery and radiotherapy The time of observation is also demonstrated

Table II *Patients which after treatment showed no signs slight signs and serious signs of ureteric obstruction*

| | Only op | Op +Ra |
|---|---|---|
| No obstruction | 39 | 15 |
| Simple obstruction | 19 | 6 |
| Serious obstruction | 6 | 15 |
| Total | 64 | 36 |

As a rule the interval between renography and urography was at most 1-2 days If the interval exceeded one week the patient was excluded from the series (in 30 cases)

matous the patient was treated postoperatively with external $^{60}$Co in a dose of 6 000 rad Patients with a stage 2a-2b tumour received 3 treatments with brachy radium before the operation as well as external X radiation or $^{60}$Co The operation was performed 1-2 months after the end of the radiotherapy Though treatment was in principle largely uniform it was nevertheless individualised The operation was performed by Wertheims method including lymph node dissection and always done by one and the same operator The patients were examined preoperatively with isotope renography urography determination of the serum creatinine and culture of the urine as well as cystoscopy and conventional routine procedures During the first 2 weeks after the operation the urine output should not fall below 1 200 ml/24 hours This sometimes required a parenteral supply of fluid The patients had an indwelling catheter for 10 days after the operation During the first 2 weeks after the operation the urine was repeatedly cultured If necessary the women were treated with appropriate antibiotics On the 14th day after the operation the patient was examined by isotope renography and intravenous urography When neither of these examinations showed anything remarkable and the patient s condition was satisfactory she was sent home The patients were reviewed after 2 4 and 6 months and in the absence of demonstrable abnormalities twice a year for the following 5 years Intravenous urography was done in the conventional way and isotope renography according to a modification of Mårtensson & Victerlov s technique (24)

## RESULTS

The results are summarised in Tables II III IV and Figs 2 and 3 In 54 patients the urinary excretion after operation appeared to be normal In 46 renography and/or urography demonstrated impaired flow on at least one occasion (Table II) In 25 of these cases the renography or urography showed signs of mild urinary stasis in the early postoperative course (Table II) The nature of such obstructions is obscure As they disappeared spontaneously within half a year they were not classified as true complications

In the remaining 21 patients the ureteric stasis was more pronounced and persisted (Table III) The women in this group had to be examined at shorter intervals and 14 of them were treated surgically due to progressive obstruction with hydronephrosis These patients fell into three groups viz stasis alone stasis with fistula stasis with recurrence (Table III) 15 of these 21 patients belonged to the group that had received combined treatment and 15 of them had a carcinoma stage 2 (Table III)

In 14% of the examinations the results of urography and renography did not agree In 13%

Table I *Age distribution of the entire material*

| Age group | 26-30 | 31-35 | 36-40 | 41-45 | 46-50 | 51-55 | 56-60 | 61-65 | 66-70 | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Only op | 2 | 2 | 12 | 15 | 10 | 15 | 8 | 0 | 0 | 64 |
| Op+Ra | 3 | 2 | 4 | 5 | 7 | 9 | 1 | 3 | 2 | 36 |
| Total | 5 | 4 | 16 | 20 | 17 | 24 | 9 | 3 | 2 | 100 |

Table III *Analysis of the patients which showed serious ureteric obstruction and the extent of the carcinoma in these patients See also text*

| | Obstr only | Obstr et fistula | Obstr et recidive |
|---|---|---|---|
| No of patients | 11 | 6 | 4 |
| Surgery necessary | 6 | 4 | 4 |
| Renal function lost (one side) | 2[a] | 0 | 0 |
| Nefrectomy | 2 | 0 | 0 |
| Ad exitus | 0 | 1 | 4 |
| Ra therapy | 7 | 4 | 4 |
| Stage I | 4 | 2 | 0 |
| Stage II | 7 | 4 | 4 |

[a] Refused plastic surgery

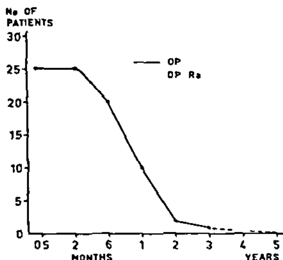



*Fig 2* Incidence of ureteric obstruction and its duration in the two groups treated with surgery only and combined treated respectively

renography was positive (showed signs of obstruction) and urography negative (normal) while in 1% urography was positive and renography negative Stasis was significantly more common on the right side ($P<0.01$) (Table IV) A comparison of the duration of ureteric obstruction in the group treated with surgery alone and that in the group given combined treatment are given in Figs 2 and 3 As seen from these figures the obstruction disappeared sooner in the former group Ureteric obstruction never occurred for the first time later than 2 months after the operation in patients treated with surgery only while in the group given combined treatment ureteric obstruction made its first appearance between 2 and 6 months after the end of treatment in 2 cases between 6 and 12 months in 2 and between 12 and 24 months in one

## DISCUSSION

It is often difficult to decide whether the urinary flow through the ureter in a given person should be regarded as obstructed or normal This is illustrated by the fact that a urogram or renogram may show signs of obstruction if the patient is examined lying but not when the patient is examined standing or after walking for a few minutes (3 27 24 36) The ureteric peristalsis also seems to vary from one individual to another (39)

In this investigation the results of postoperative urography and renography were strictly compared with those obtained before the operation An attempt was made to grade the degree of obstructor Each patient was her own control Table III shows that the urinary transport through the ureter was impaired in 21 patients In 4 of them the ureteric obstruction was due to compression by a tumour this means that the frequency of obstruction as a real complication of treatment was 17%

Obstruction in patients treated for carcinoma of the uterine cervix examined with intravenous urography alone varies between 7 and 18% (5 12 19 23 29) Soiva & Rynanen (37) 1969 demonstrated postrenal obstruction in 16 of 48 pa

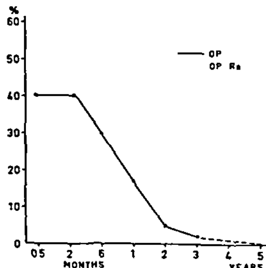



*Fig 3* The percentage incidence of ureteric obstructions of the patients in Fig 2

Table IV *Side of obstruction in the patient which after teratment showed signs of obstruction*

| | Right | Left | Bilat | No of patients |
|---|---|---|---|---|
| Serious | 5 | 5 | 11 | 21 |
| Simple | 14 | 2 | 9 | 25 |
| Total | 19 | 7 | 20 | 46 |

tients operated for carcinoma of the uterine cervix In a thorough investigation reported in 1972 by Mayer et al (27) obstruction was found in 19 of their 34 patients

The relatively high incidence of postrenal obstruction in the present investigation may be explained partly by the close follow up and partly by the high sensitivity of renography The patients were not systematically examined for vesico ureteric reflux The possible effect of this complication as well as disturbances in micturition will be studied in a later investigation The investigation clearly shows that postrenal obstruction after surgical or combined treatment of carcinoma of the uterine cervix is more common than hitherto supposed It also demonstrates that the majority of the early postoperative ureteric obstructions in the group of patients treated with surgery alone soon disappear (Figs 2 and 3) This observa on is in accordance with previous reports (37 3 14 20 10) The nature of such early is obscure They have been ascribed o postoperative oedema accentuated by impairment of lymph flow and urinary infection or to denervation of the urinary bladder and the lowest segment of the ureter at the operation (15 10 25 7) But the demonstration of postoperative denervation of the ureter requires assessment of ureteric function immediately after the operation Ureteric obstructions which occur in the later course (>½ year postoperatively) are usually caused by fibrosis or recurrence of the tumour

As in earlier investigations (2 27 37 10) severe obstruction was found to be more common after combined treatment (4) It is difficult to estimate to what extent the obstruction in a given patient should be ascribed to surgery or to radiotherapy respectively In a large series of women treated with radiation for carcinoma of the uterine cervix Slater & Fletcher (35) found that ureteric stricture after radiotherapy alone was uncommon This view is shared also by Kottmeier (21) while Gansau (12) Monch & Halltorff (30) claim that strictures are more common after radiotherapy than after operation According to Benson & Hinman (1) however strictures are more common after surgery alone

The significance of tumour infiltration of the parametrium in the development of later ureteric stasis (later after hysterectomy) has been debated in the literature (4 30 22) This investigation shows a clear correlation between the extent of the carcinoma and later serious impairment of urinary flow through the ureter (Table III) This is in variance with Mayer et al (27) The obstruction persisted longer in the patients that had received combined treatment (Figs 2 and 3) Moreover postrenal obstruction not due to a recurrence occurred for the first time after more than one year in the group that had received combined treatment In the group treated with surgery alone post renal obstruction never occurred for the first time later than 6 months after the operation This accords with earlier reports (6 9 20 25) Table III summarised the serious obstructions found in 6 of the cases there was coexisting fistula and in 4 the obstruction was due to a recurrence of the tumour In 2 the recurrence was demonstrated first by renography and confirmed by urography and not until 2-3 months later could any tumour change be palpated Postrenal obstruction has been described as an early sign of recurrence also by other authors (10 37) and underlines the value of close postoperative urinary output controls with renography and/or urography

As pointed out by Lundgren et al (24) renography may be used as a screening method for detecting postoperative postrenal obstruction Should the renogram prove signs of obstruction the patient should be examined with urography to visualise the nature and significance of this obstruction

As in previous reports (13 23 37) postrenal obstruction was significantly ($p<0.01$) more common on the right side (Table IV) Nos satisfactory explanation can be offered for this phenomenon During pregnancy the common right side ureteric obstruction (31 34) has tentatively been ascribed to compression of the ureter owing to dilatation of the right ovarian vein (18) It has however been shown in monkeys that excision of

*the ovarian vein after development* of ureteric dilatation during pregnancy has no effect on the course of the dilatation (33) No relation was found in the present series between dilatation of the right ureter and removal or conservation of the right or left ovary

Patients with severe postrenal obstructions were treated with percutaneous nephropyelostomy (38) The valuable effect of an early pyelostomy on a hydronephrotic kidney in such patients must be stressed (17) In our patients percutaneous nephropyelostomy gave good results (38) It saved renal function in 12 patients In 5 of these the ureteric obstruction disappeared without other therapy In the remaining patients plastic surgery was necessary In 4 cases the ureter was reimplanted to the urinary bladder in 2 patients it was implanted to a loop of the small intestine and in 1 case it was transposed to the other ureter

Thanks to the careful postoperative follow up *early relief of the pressure* on the kidney and plastic surgery in a quiescent stage only 2 patients were subjected to nephrectomy despite the relatively high incidence of stasis Two patients refused operation Percutaneous nephropyelostomy made a separate renal function test possible (PAH-clearance) and the function of both the kidneys could be estimated

The present investigation shows that only by close follow up with renography and urography it is possible to detect whether a postrenal obstruction tends to regress or progress If it progresses the pressure on the kidney may be relieved by a pyelostomy and later in a quiescent stage surgical treatment can be undertaken Such procedures can probably reduce the frequency of serious renal dysfunction after treatment of carcinoma of the uterine cervix

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*Submitted for publication Febr 19 1974*

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Acta Obstet Gynecol Scand 54 303–305 1975

# RELATIONSHIP BETWEEN ENDOMETRIAL ARIAS STELLA PHENOMENON AND CONCENTRATIONS OF URINARY CHORIONIC GONADOTROPIN AND SERUM HUMAN PLACENTAL LACTOGEN

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*Abstract* Serum placental lactogen (HPL) and urinary chorionic gonadotrophin (HCG) were measured in 14 patients with the endometrial Arias-Stella phenomenon and in 35 comparable patients without this reaction in order to explore a possible causative relationship between the hormonal levels and the endometrial changes The concentrations of HPL and HCG were similar in both groups of patients indicating a lack of correlation between the levels of these two hormones and endometrial changes

Focal changes in endometrium from cases of uterine abortion hydatidiform mole and chorionic carcinoma were first described by Arias Stella (1) Similar endometrial changes have been reported in varying proportions of cases of these conditions and of ectopic pregnancy (2 3 6 8) The cause of this reaction is disputed but Beswick & Gregory (3) speculated that the changes might result from alterations in hormonal balance due to disturbance of the trophoblast

The present study was designed to study the possibility that imbalance in the output of the two peptide hormones synthesized by syncytiotrophoblastic cells plays a part in the etiology of the Arias Stella phenomenon

## MATERIAL AND METHODS

From 49 patients whose pregnancies ended unsuccessfully after vaginal bleeding serum HPL and urinary HCG concentrations were measured on the first day in hospital The mean duration of pregnancy calculated from the first day of the last menstrual period was 11 4 weeks with range of 7 to 16 weeks Curettage was performed 1–3 days after beginning of the vaginal bleeding The tissue obtained at evacuation was fixed in buffered 10% formal saline and 5 $\mu$ thick paraffin sections were made and stained with haematoxylin and eosin In 14 cases including one ectopic pregnancy focal endometrial changes as described by Arias Stella (1) were observed (Fig 1) In 35 cases the endometrium was normal or showed some pathology but no Arias Stella reaction The mean duration of pregnancy in patients with the Arias-Stella atypia was 9.5 weeks (range 7–16 weeks) and in patients without this atypia 1 1 weeks (range 8–16 weeks)

Serum HPL was determined by a double antibody radioimmunoassay using the HCS Sclavo-Sorin kit (Saluggia Italy) (4) The determinations were made in duplicate and the variation was 8 0% Twenty four hour urinespecimens were collected during the first day in hospital The HCG content was measured semiquantitatively by the test of Pregnosticon (Organon Holland) The sensitivity limit of this test is 1500 IU/l urine

## RESULTS

The concentrations of HPL and HCG in each patient are shown in Fig 2 The means of HPL and HCG levels in cases with or without the Arias-Stella phenomenon are not calculated because the durations of the pregnancies varied The distribution of the patients with the Arias Stella phenomenon was not different from the cases without this abnormality The patients can be divided into two groups by a line where the relative increases in the concentrations of HPL and HCG were the same (Fig 2) Eight out of 28 cases above the line and six out of 21 cases below the line had the Arias Stella reaction

*Fig 1* Endometrial Arias Stella reaction showing intraluminal budding enlargement and vacuolation of cells with slight nuclear hyperchromasia and hyper trophy Haematoxylin and eosin ×350

## DISCUSSION

The diagnosis of ectopic pregnancy before the of an abdominal catastrophe may be icult partly because the pregnancy tests are often negative Synthesis and release of HCG from ectopic trophoblastic tissue is diminished (11) but maternal circulating HPL levels in ectopic pregnancies are normal (9) Because the Arias Stella phenomenon is commonly associated with ectopic pregnancies (2 3 6) it might be thought that the dominance of HPL over HCG in ectopic pregnancy plays a part in the etiology of the phenomenon but our results do not support this

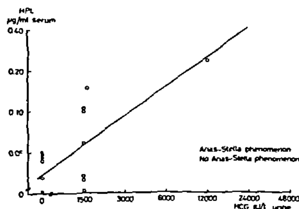



*Fig 2* Distribution of the patients according to the concentrations of serum HPL and urinary HCG Eight out of 28 cases above the line and six out of 21 cases below the line had the endometrial Arias-Stella reaction

In molar pregnancies serum HPL levels have been reported to be lower but HCG output higher than normal (7 10) We did not have any cases of molar pregnancy but the dominance of HCG over HPL without hydatidiform degeneration was not related to the occurrence of Arias Stella reaction

Infiltration of the cytoplasm with glycogen seems to be the cause for the appearance of cells in the Arias Stella phenomenon (3) As HPL may play a role in the metabolism of carbohydrates and fats in pregnancy (5) it is possible that gly cogen infiltration and HPL levels are related to each other However in this study the Arias Stella phenomenon was not related to the absolute HPL level

## ACKNOWLEDGEMENT

The authors gratefully acknowledge Dr Frej Stenback for his help This study was supported by a grant from the Emil Aaltonen Foundation Tampere Finland

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*Submitted for publication Febr 22 1974*

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Acta Obstet Gynecol Scand 54 307–313 1975

# STUDIES IN CHOLESTASIS OF PREGNANCY

## IV *Serum Lipids and Lipoproteins in Relation to Duration of Symptoms and Severity of the Disease and Fatty Acid Composition of Lecithin in Relation to Duration of Symptoms*

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*Abstract* Pregnant women with pruritus and with cholestasis of pregnancy verified from the medical history and by the presence of lipoprotein-X in serum have been studied Thirty nine patients were investigated for serum lipids (cholesterol phospholipids and triglycerides) serum lipoproteins (high density lipoproteins cholesterol and estimated low-density lipoproteins cholesterol) and for hematological data (serum iron and serum iron binding capacity) in relation to duration of pruritus In 78 patients the serum lecithin concentrations and fatty acid compositions were also analysed Severity and duration of the disease appeared to influence the lipid/lipoprotein metabolism The results support the hypothesis of an abnormal reaction of liver metabolism to estrogens in the initial stage of cholestasis of pregnancy

Cholestasis of pregnancy (CP) occurs in predisposed individuals in different degrees of severity In its milder form (pruritus gravidarum=PG) it is characterized by generalized pruritus and minimal changes in liver function tests and in its more severe form (hepatosis of pregnancy=HP) elevations of serum transaminases (SGOT and SGPT) with or without jaundice Morphological studies in liver biopsy specimens reveal changes typical of cholestasis in general in both stages of the disease (1 7 26)

Cholestasis of pregnancy is considered to be hormonally induced It is suggested that estrogens are primarily responsible for provoking impaired hepatic excretory function An abnormal reaction to normal amounts of estrogens produced during pregnancy rather than abnormally high level of estrogens appears to be the cause of CP (2 45) The same alterations in liver function occur to a smaller extent during normal pregnancy and during treatment with natural estrogens (29 39)

Lipids in serum are transported as macromolecular compounds the lipoproteins The four classes of serum lipoproteins with characteristic size and density are the chylomicrons the very low-density lipoproteins (VLDL) the low-density lipoproteins (LDL) and the high-density lipoproteins (HDL) The major lipid constituents of lipoproteins are free cholesterol cholesterol esters triglycerides and phospholipids The phospholipids are composed of sphingomyelin and three major phosphoglyceride fractions lecithin (phosphatidyl choline) cephalin (phosphatidyl-ethanolamine) and lysolecithin

In a previous paper (23) it has been reported that in hepatosis of pregnancy (HP) serum lipids i e cholesterol phospholipids triglycerides and estimated LDL are increased and HDL decreased The elevations during cholestasis of cholesterol and phospholipids are mainly due to the occurrence of a pathological lipoprotein LP X within the LDL density class (37 38) We have earlier presented evidence that the occurrence of LP X in serum can be used as a sensitive diagnostic criterion in cholestasis of pregnancy (20 22)

In the present investigation patients with different degrees of severity of CP and with different durations of pruritus have been studied in relation to serum lipids and lipoproteins to hematological data and to serum lecithin fatty acids in order to trace possible variations in the influence of pregnancy and cholestasis on liver metabolism

## MATERIALS AND METHODS

During a two-year period consecutive pregnant women complaining of generalized pruritus were admitted at the Maternity Welfare Unit Sahlgren s Hospital The diagno-

Table I *Serum lipids lipoproteins and hematological data in pruritus gravidarum (PG) and hepatosis of pregnancy (HP) in relation to duration of pruritus compared to control series*
Mean±S E M N S =not significant

| | | | Serum cholesterol (mg/100 ml) | Serum triglycerides (mg/100 ml) | Serum phospholipids (mg/100 ml) | HDL cholesterol[a] (mg/100 ml) | LDL cholesterol[b] (mg/100 ml) |
|---|---|---|---|---|---|---|---|
| I | PG short duration ≤2 weeks $n$=7 | $\bar{x}$ | 252 | 168 | 293 | 87 | 131 |
| | | S E M | 6 | 19 | 12 | 5 | 5 |
| II | PG long duration >2 weeks $n$=11 | $\bar{x}$ | 266 | 216 | 304 | 66 | 150 |
| | | S E M | 15 | 29 | 15 | 6 | 16 |
| III | HP short duration ≤2 weeks $n$=12 | $\bar{x}$ | 351 | 278 | 386 | 67 | 212 |
| | | S E M | 19 | 11 | 22 | 4 | 17 |
| IV | HP long duration >2 weeks $n$=9 | $\bar{x}$ | 293 | 250 | 315 | 50 | 182 |
| | | S E M | 24 | 23 | 27 | 3 | 19 |
| V | Control series $n$=20 | $\bar{x}$ | 265 | 180 | 283 | 77 | 147 |
| | | S E M | 8 | 13 | 7 | 3 | 8 |
| | I vs V | $P<$ | N S | N S | N S | N S ($t$=1.5) | N S |
| | II vs V | $P<$ | N S | N S | N S | N S | N S |
| | III vs V | $P<$ | 0.01 | 0.001 | 0.001 | N S | 0.01 |
| | VI vs V | $P<$ | N S | 0.01 | N S | 0.001 | N S |

[a] Measured value [b] Estimated value

sis of cholestasis of pregnancy (CP) was verified through a typical medical history and by the presence in serum of LP X (22) The patients were subdivided according to earlier definition (22) using the following values in liver function tests Pruritus gravidarum (PG) serum bilirubin <1.2 mg/100 ml and SGOT and SGPT <50 Units/l Hepatosis of pregnancy (HP) serum bilirubin ≥1.2 mg/100 ml and SGOT and SGPT ≥50 Units/l The patients have been further subdivided according to the duration of their pruritus Pruritus ≤2 weeks was considered to be of short duration and >2 weeks of long duration

*Patient series for studies of lipids lipoproteins and hematological data* Thirty nine arbitrarily chosen patients have been subdivided in the following groups

1 PG short duration $n$=7 (gestational age mean 37.1 weeks)
2 PG long duration $n$=11 (gestational age mean 33.3 weeks)
3 HP short duration $n$=12 (gestational age mean 36.0 weeks)
4 HP long duration $n$=9 (gestational age mean 34.4 weeks)

*Patient series for studies with gas liquid-chromatography (GLC)* Twenty eight patients were chosen at random for studies with GLC in fatty acid composition of lecithin Subdividing this patient series in the same manner as the former series give too small groups and thus subdivision in cholestasis of pregnancy (CP) with short and long duration is more convenient

Table II *Relative fatty acid composition (mole per cent) of lecithin in women with cholestasis of pregnancy (CP) in relation to duration of their pruritus*
N S =not significant

| | | | 16:0 | 16:1 ($n$-7) | 18:0 | 18:1 ($n$-9) | 18:2 ($n$-6) | 20:3 ($n$-6) | 20:4 ($n$-6) |
|---|---|---|---|---|---|---|---|---|---|
| I | CP short duration ≤2 weeks $n$=10 | $\bar{x}$ | 35.1 | 1.3 | 10.5 | 13.8 | 21.7 | 3.3 | 7.4 |
| | | S E M | 0.83 | 0.12 | 0.44 | 0.57 | 0.98 | 0.15 | [illegible] |
| II | CP long duration >2 weeks $n$=18 | $\bar{x}$ | 34.5 | 1.3 | 9.9 | 14.8 | 25.7 | [illegible] | 5.4 |
| | | S E M | 0.50 | 0.08 | 0.37 | 0.55 | 0.75 | 0.15 | [illegible] |
| III | Control series $n$=20 | $\bar{x}$ | 37.4 | 1.0 | 9.5 | 12.7 | 24.9 | 2.8 | 5.7 |
| | | S E M | 0.42 | 0.04 | 0.22 | 0.25 | 0.44 | 0.14 | 0.25 |
| | I vs III | $P<$ | 0.01 | N S | 0.05 | N S | 0.01 | 0.05 | 0.001 |
| | II vs III | $p<$ | 0.001 | 0.05 | N S | 0.01 | N S | N S | N S |

| g/100 ml) | Serum iron (µg/100 ml) | LP X (arb units) |
|---|---|---|
| | 103 | 2 0 |
| ?5 | 17 | 0 3 |
| 7 | 106 | ? 1 |
| 6 | 18 | 0 2 |
| | 155 | 3 ? |
| 40 | 20 | 0 2 |
| 11 | 135 | 3 3 |
| 46 | 19 | 0 3 |
| | 100 | – |
| 18 | 8 | |
| S | N S | – |
| 001 | N S | – |
| 05 | 0 05 | – |
| 01 | N S | – |

1 CP short duration $n=10$ (gestational age mean 34 9 weeks)

? CP long duration $n=18$ (gestational age mean 3? 7 weeks)

Twenty women with uncomplicated pregnancies (gestational age mean 33 6 weeks) chosen at random from the same Maternal Welfare Unit served as a control series

*Blood sampling and chemical methods*

Blood samples were drawn in the fasting state the morning after clinical examination Samples were analysed for liver function tests serum total bilirubin (normal <1 2 mg/100 ml) alkaline phosphatase (normal <8 Buch Units) SGOT (normal <17 Units) and SGPT (normal <17 Units/l) performed at the Laboratory of Clinical Chemistry according to standard methods Serum triglycerides were determined according to Carlson (11) total cholesterol by the method of Cramer & Isacsson (12) and phospholipids as described by Svanborg & Svennerholm (4 ) The cholesterol content (?5) of high-density lipoproteins (HDL) was measured after the precipitation of very low density lipoproteins (VLDL) and low-density lipoproteins (LDL) with manganese chloride and heparin (9) The cholesterol content of LDL was estimated from the equation LDL cholesterol=serum cholesterol—HDL cholesterol—serum triglycerides×$F$ As $F$ the value 0 ?0 was used for serum triglycerides <180 mg/100 ml and the value 0 25 for serum triglycerides >180 mg/100 ml (17)

The semiquantification of LP X was performed as described earlier ( 2) by a modified immunodiffusion technique The hematological variables studied were serum iron and serum iron binding capacity (TIBC)

| 6 ) | 18–2? ($n-6$) | 18 2/ ?0 4 | Lecithin (mg/100 ml) |
|---|---|---|---|
| | 3? 9 | 3 0 | ?61 2 |
| 6 | 0 69 | 0 19 | ?1 5 |
| ? | 34 5 | 4 9 | ?74 9 |
| 28 | 0 7? | 0 33 | 9 0 |
| | 33 7 | 4 4 | 259 4 |
| ?4 | 0 48 | 0 28 | 6 9 |
| | N S | – | N S |
| S | N S | – | N S |

*Gas liquid-chromatography (GLC) procedure*

*Blood samples* were centrifuged at 2500×$g$ for ten minutes and the serum recovered immediately frozen and stored at −20°C in glass tubes with teflon screw caps

*Chemicals* All solvents (reagent grade) used in the fatty acid analyses were redistilled before use Other chemicals were of analytical quality

*Preparation of lipid extract* Preparation of lipid extract separation of lipids by thin layer chromatography on Silica gel and isolation of lecithin and phosphoglyceride spots and preparation of fatty acid methyl esters were performed as described earlier (30)

*GLC of methyl esters* GLC of methyl esters measurement of lecithin fatty acids and conversion from weight per cent to mole per cent were performed as described earlier (?1)

Except the fatty acids presented in the tables 14 0 15 0 18 3 ($n-6$) 18 3 ($n-3$)+?0 1 ($n-9$) ?0 3 ($n-9$) 2? 4 ($n-6$) 2? 5 ($n-6$) and 2? 5 ($n-3$) have been identified (43) but not tabulated because their concentrations were generally less than one per cent

*Measurement of serum lecithin* For measurement of serum lecithin from fatty acid content in lecithin (obtained from GLC) the equation $y=1\ 66x+75$ has been used (?1) where $y$ is the content of lecithin in serum and $x$ is the fatty acid in serum lecithin which was determined

*Statistical methods*

Conventional methods were used for the calculation of means standard deviation and standard error of means Student s $t$ test was used to study differences between groups Values of $p<0\ 05$ were considered statistically significant (8)

## RESULTS

All comparisons are made in relation to control series

*Serum lipids* (Table I) Serum cholesterol ($p<0\ 01$) and phospholipids ($p<0\ 001$) were elevated in the severe form of cholestatic pregnancy (HP) of short duration Serum triglycerides were highest in HP—

Table III *Concentrations (mg/100 ml) of serum lecithin with linoleic acid (18 2) in 2 position and serum lecithin with arachidonic acid (20 4) in 2 position in two groups of cholestasis of pregnancy (CP) with short and long duration of pruritus compared to normal pregnancy*

N S = not significant

| | | | 18 2 lecithin (mg/100 ml) | 20 4 lecithin (mg/100 ml) | 18 2 lecithin/ 20 4 lecithin ratio |
|---|---|---|---|---|---|
| I | CP short duration | x̄ | 55 7 | 19 4 | 2 9 |
| | ≤2 weeks $n=10$ | S E M | 3 5 | 1 9 | – |
| II | CP long duration | x̄ | 70 7 | 14 9 | 4 7 |
| | >2 weeks $n=18$ | S E M | 3 2 | 0 9 | – |
| III | Control series | x̄ | 64 5 | 14 9 | 4 3 |
| | $n=20$ | S E M | 2 0 | 0 8 | – |
| | I vs III | $P<$ | 0 05 | 0 05 | – |
| | II vs III | $P<$ | N S | N S | – |

of short duration ($p<0\ 001$) and remained elevated in HP of long duration ($p<0\ 01$)

*Serum lipoproteins* (Table I) HDL-cholesterol (expression for HDL lipids) was characteristically low in HP of long duration ($p<0\ 001$) The estimated LDL cholesterol was elevated ($p<0\ 01$) in HP of short duration concomitant with the appearance of large amounts of LP X

*Hematological data* (Table I) Serum iron was elevated by 55 % in HP of short duration ($p<0\ 05$) The mean value for TIBC was high in PG of long duration ($p<0\ 001$) in HP of short duration ($p<0\ 05$) and even higher in HP of long duration ($p<0\ 01$)

*Serum lecithin relative fatty acid composition* (Table II) Palmitic acid (16 0) was low ($p<0\ 01$) in cholestasis of short duration and even lower ($p<0\ 001$) after longer duration Oleic acid (18 1 ($n-9$)) was high ($p<0\ 01$) in cholestasis of long duration Arachidonic acid (20 4 ($n-6$)) was characteristically high ($p<0\ 01$) in cholestasis of short duration while linoleic acid (18 2 ($n-6$)) was low ($p<0\ 01$)

The molar ratio linoleic/arachidonic acid (18 2/20 4) was low (3 0) after short duration and high (4 9) after long duration

The above differences between CP short and normal pregnancy based on relative amounts of 18 2 and 20 4 remain when calculated on an absolute basis i e when based on the absolute amounts of 18 2 lecithin and 20 4 lecithin 18 2 lecithin was low ($p<0\ 05$) and 20 4 lecithin high ($p<0\ 05$) in CP of short duration The 18 2/20 4 ratios remained at similar levels as those calculated on relative basis (Table III)

## DISCUSSION

In earlier reports (21 23) we have shown elevated serum cholesterol in the severe form of cholestasis of pregnancy (hepatosis of pregnancy=HP) in accordance with other authors (3 31 34) and elevated phospholipids (3) and low-density lipoproteins (LDL) (3) as well as decreased high-density lipoproteins (HDL) (3) In addition we have disclosed (23) increased serum triglycerides and lower serum lecithin fatty acid content of 16 0 (palmitic acid) and higher content of 18 1 (oleic acid) (21)

In the present publication patients ($n=39$) studied for serum lipids lipoproteins and hematological data were subdivided into groups of different degrees of severity (PG and HP) and duration of symptoms This was done to elucidate whether influences on lipid metabolism were related to these parameters

In the mild form (PG) with short duration ( PG short ) a tendency to increased HDL was observed concomitant with the occurrence of lipoprotein X As cholestatic conditions usually are characterized by decreased HDL (15 33 37 44) this particular finding in the initial phase of CP might suggest another metabolic influence on the lipoprotein metabolism Earlier indications (23) have pointed to an increased influence of estrogens in relation to CP An increase in HDL would support this suggestion

In the mild form (PG) with longer duration ( PG long ) HDL decreased possibly indicating an increased influence of cholestasis per se

In the more severe form (HP) with short history ( HP short ) the most striking alterations in serum lipids and lipoproteins occurred i e elevations in all serum lipids and in LDL These changes probably are expressions of the cholestatic condition itself The characteristic serum lipid changes during cholestasis are an increase in free cholesterol and phospholipids due preferentially to the appearance of cholesterol and phospholipid rich LP X within the LDL class (37) The primary cause for an increase in cholesterol in cholestasis is an influence on the bile acid feed back mechanism in the liver In the present situation with increased bile acids (cholic and chenodeoxycholic acid preferentially) (40) the conversion of cholesterol to bile acids is depressed causing increased levels of cholesterol in the liver and serum (28)

Serum triglycerides on the other hand are usually not increased in cholestatic conditions (14 32 37) The high serum triglyceride level in the present series appears therefore not to be explained by cholestasis The administration of estrogens causes increased serum triglycerides as well as phospholipids and HDL (6 13 18 19 24) It is therefore tempting to link the elevated serum triglycerides in PG long as well as HP short to an increased estrogen influence on the triglyceride synthesis and/or removal (19)

In the severe form (HP) of longer duration ( HP long ) there was a decreasing pattern in all serum lipids and a further decrease in HDL In an earlier report (23) correlations between elevated (>50 Units/l) SGOT values and a decrease in serum triglycerides were found Liver parenchymal cell damage therefore appears to be a possible explanation for the reduction in lipids in the long standing severe form of CP

In the present series there was also a tendency towards increased values of TIBC in relation to duration and severity of cholestasis Serum iron on the other hand was most elevated in HP short possibly indicating an outflow of iron from the liver parenchymal cells preferentially in the initial stage of the cell damage (41)

In the series studied with respect to lecithin fatty acids the limited number of patients allowed only a subdivision in relation to duration of the disease In CP of short duration ( CP short ) linoleic acid (18 2) content decreased ($p<0\ 01$) and arachidonic acid (20 4) increased ($p<0\ 001$) in serum lecithin and the molar ratio between these two fatty acids (18 2/20 4) was low (3 0) as compared to control series (4 4) In CP long this ratio (18 2/20 4) again returned to a higher value (4 9) These differences are further supported by the fact that they remain significant when determined on an absolute basis (Table III)

The fatty acid composition in liver and serum lecithin is determined by the synthetic pathways of lecithin in the liver The faster and quantitatively dominating cytidine diphosphate diglyceride pathway *Pathway I* (the Kennedy pathway) (35) causes the appearance of lecithin with 16 0 in 1 position and 18 1 or 18 2 in 2 position while *Pathway II* with methylation of phosphatidyl ethanolamine (cephalin) (36) preferentially causes the appearance of lecithin with 18 0 in 1 position and 20 4 in 2 position (16 36) Thus the variations in fatty acid pattern of serum lecithin in the present study would suggest an enhancement of Pathway II in the early phase of CP ( CP short ) possibly by the influence of estrogens (27) while in the more advanced cholestasis ( CP long ) Pathway I is enhanced possibly under the influence of bile acids (4 5) This latter finding is in agreement with in vitro studies showing higher utilization of Pathway I with a higher incorporation of precursors into lecithin in the presence of bile acids (5 10)

The present data on the fatty acid composition of serum lecithin also reveal a reduction independent of severity or duration of cholestasis in 16 0 (palmitic acid) in CP This finding is not readily explained at the present time

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*Submitted for publication Febr 23 1974*

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Acta Obstet Gynecol Scand 54 315–318 1975

# INFLUENCE OF COPPER INTRAUTERINE CONTRACEPTIVE DEVICES (Cu 7 IUD) ON THE MENSTRUAL BLOOD LOSS

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*Abstract* In a series of 43 healthy women menstrual blood loss was determined before and after insertion of a copper IUD (Gravigard) The menstrual blood loss before insertion was compared with the blood loss 1 2 3 4 5 6 7 and 12 months after insertion An increase amounting to approximately 20 ml per period without significant variations during the study was recorded No significant influence upon serum iron and TIBC was found in 15 of the subjects selected by random. Compared with plastic IUD s the tested copper IUD causes a less pronounced increase of the menstrual blood loss The menstrual blood loss in a small group of women with menorrhagia was determined before and up to 5 months after insertion of copper IUD No aggravation of the menorrhagia was however recorded in this group

In accordance with other contraceptives the use of intrauterine devices may be accompanied by certain side effects Thus distrubances of the menstrual bleeding pattern may occur According to Lippes (6) around 90% of women using Lippes loop exhibit some alterations of their menstrual pattern most pronounced during the first months after the insertion Such side effects may result in removal of the IUD and in previous reports the IUD was removed because of bleeding abnormalities during the first year after insertion in 5–16% of the women studied (1 10)

The alterations of the menstrual bleeding pattern caused by IUD s are predominantly of two types One is small uterine bleedings occurring between the periods ( spotting ) and the other type is heavy menstrual blood loss during the period—menorrhagia In studies where the menstrual blood loss was measured objectively the increase in the menstrual blood loss was as an average 82–106% during the first three months after insertion of Lippes loop® or Saf T-coil® 33 S (2 10) Such an increase of the blood loss may in some women be followed by iron deficiency

In a population study of the menstrual blood loss Hallberg et al (3) found a median value of 30 ml and when the blood loss exceeded 80 ml per period the risk for iron deficiency was considerable The increase of the blood loss due to the use of IUD s may therefore be associated with such a risk especially in populations in which the iron balance situation is critical because of a low dietary intake of iron Previous studies on the effect of IUD s on the menstrual blood loss were performed with the old types of IUD—e g Lippes loop® or Saf T-coil® By the introduction of the new and considerably smaller copper IUD it has been assumed that the frequency of some side effects should diminish and it has also been supposed that heavy menstrual blood losses should be less frequent

The aim of the present study was to quantify the menstrual blood loss before and after the insertion of one of the most commonly used copper IUD s (Cu 7 IUD Gravigard®) The study has been extended for one year to make it possible to follow the variation of the menstrual blood loss during a relatively long period and to study the effect of the alterations of the blood loss on some parameters reflecting the iron balance

## MATERIAL AND METHODS

One type of copper IUD Cu 7 Gravigard® with a copper surface of 200 mm² was used throughout the study Forty-three healthy women with normal menstrual blood losses and four women with menorrhagia (i.e blood loss above 80 ml per period) were included in the study All subjects were studied with reference to the menstrual blood loss before and after the insertion of IUD The mean age of

Table I *Menstrual blood loss (ml) before and after insertion of IUD (Cu 7)*

| | Control periods | | Periods after insertion | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 3 | 4 | 5 |
| No of subjects | 43 | 42 | 39 | 36 | 29 | 27 | 25 |
| Mean menstrual blood loss ±S E.M | 38 2±2 7 | 34 3±1 4 | 54 3±4 1 | 58 6±4 6 | 58 4±5 4 | 58 8±5 5 | 55 9±4 |
| Mean increase* ±S E.M | – | – | 18 0±3 1 | 22 3±3 1 | 22 1±3 8 | 2?.5±3 2 | 19 6±3 |
| Increase in percent of control periods | – | – | 50 | 61 | 61 | 62 | 54 |
| Mean Hb conc g/100 ml | 13 1±0 1 | 13 3±0 2 | 13 3±0 1 | 13 4±0 2 | 13 2±0 2 | 13 0±0 ? | 13 0±0 |

The increases are in all groups statistically significant ($p<0\ 001$)

the 43 women with normal blood losses was 29 years (range 21–42 years) All were parous women Fourteen women had one child 19 had two five had three children and five women had given birth to four children Age and parity of the four menorrhagic women appear from Table III

Prior to the study each patient was interviewed and a general and a gynecological examination was made No patients with gynecological disease were included About half of the women had used oral contraceptives before entering the study In two subjects the pills were withdrawn only two months before the start of the study—in all other cases *this interval was considerably longer*

The blood loss was determined according to the method devised by Hallberg & Nilsson (4) Each woman was carefully instructed how to collect her menstrual blood In order to avoid waste of blood both a sanitary towel a tampon was used The towels and tampons were ... at home in a plastic container with tightly fitted er

The hemoglobin in the sanitary towels and tampons was extracted with 1 25 M NaOH as alkaline hematine which was then determined spectrophotometrically The amount of the menstrual hemoglobin was expressed as ml blood by

Table II *Menstrual blood loss concentration of hemoglobin serum iron and TIBC in 15 women before and approximately one year after insertion of IUD (Cu 7)*

| | Before insertion | One year after *insertion* | Significance |
|---|---|---|---|
| Mean menstrual blood loss ±S E.M | 31 4±4 8 | 57 9± 8 5 | $p<0\ 0?$ |
| *Hemoglobin conc* g/100 ml blood ± S E.M. | 13 1± 0 1 | 13 0± 0 2 | NS |
| Serum iron (µg/ 100 ml blood) ± S E.M | 109 6±11 6 | 112.1±11.8 | NS |
| TIBC (µg/100 ml blood) ±S E.M | 364 6±12 2 | 360 8±18 9 | NS |

using the hemoglobin concentration in venous blood determined as cyanmethemoglobin The methods for determination of serum iron and total iron binding capacity (TIBC) were those used in the routine work in the hospital laboratory Before insertion of the IUD the menstrual blood loss was determined at two consecutive periods During the following seven months the blood loss was measured and side effects possibly linked to the IUD was registered by interviews Due to various reasons all women were not able to collect their menstrual blood at each period The number of participants each month appear from Table I In 23 women the menstrual blood loss was measured roughly one year (range 11–14 months) after the insertion of the IUD The statistical differences between groups were calculated according to Student s *t* test.

## RESULTS

Table I summarizes data from the participants in the group with normal menstrual blood loss The mean menstrual blood loss and the mean increase of the blood loss are *indicated in* the table The mean increase during the first seven months was approximately 20 ml per period corresponding to an increase of 55% of the blood loss in comparison to the control periods This increase is statistically significant ($p<0\ 001$) The blood loss one year after the insertion was of the same magnitude as during the first seven months

In women with a blood loss below 80 ml per period before the insertion of the IUD a total of 193 periods were measured after the insertion In 36 out of these 193 periods (18 8%) the blood loss exceeded *80 ml* which according to previous reports (3) is the upper limit above which most women develop iron deficiency Such heavy periods were distributed among 13 women but out of these there was only one whose blood loss exceeded 80 ml every period after the

| | | |
|---|---|---|
| | 7 | ≈1? |
| 5 | 19 | 23 |
| 8 8±6 6 | 53 3±6 5 | 57 1±5 3 |
| ? 5±5 2 | 17 0±4 8 | ?0 8±3 3 |
| ? | 47 | 57 |
| 3 3±0 2 | 13 2±0 2 | 13 1±0 1 |

insertion In six women this level was reached approximately every second period

In one women a decrease of 1 7 g hemoglobin per 100 ml blood was found after six months but in the group followed for one year no decrease amounting to more than 1 5 g hemoglobin per 100 ml blood was recorded Furthermore in 15 women the serum iron concentration and the total iron binding capacity (TIBC) were determined before and one year after the insertion of the IUD No significant change of these parameters were found while the increase of blood loss after insertion was statistically significant (Table II)

Table III shows data from four patients with menorrhagia prior to the insertion of the IUD In 16 periods the blood loss was measured after the insertion It is interesting to notice that in 12 of these periods the blood loss decreased and in the remaining 4 periods the increase of the blood loss was relatively small compared with women with normal menstrual blood loss

## DISCUSSION

In the present study the mean menstrual blood loss increased with approximately ?0 ml per period after the insertion of the copper IUD corresponding to approximately a 55% increase above the control level Westrom & Bengtsson (10) found an average increase of 29 6 ml (82 7%) with older types of IUDs without copper (Lippes loop® and Saf T-coil®) In a study by Guttorm (2) an average increase of 37 6 ml (106%) of the menstrual blood loss was found when a Gynekoil® was used It should be emphasized that the mean blood loss in the control periods is almost the same in these two above mentioned studies as in the present investigation The method for determination of the menstrual blood loss was also identical (4) Thus it is clear that the smallest increase was found by use of the copper 7 IUD The results of the present study also show that the blood loss is of the same magnitude during the first months after the insertion as after one year Therefore it seems reasonable to assume that the menstrual blood loss is comparatively constant at a higher level as long as the IUD is in position in the uterus

It is evident that an increase of the menstrual blood loss may lead to an increased risk for development of iron deficiency In previous reports (3 8) it has been shown that when the blood loss amounts to more than 80 ml per period the frequency of iron deficient subjects is high In the present study (Table I) it was found that only one woman regularly had a blood loss above 80 ml after insertion of the IUD In some other women this level of menstrual blood loss was reached occasionally Furthermore no significant changes were observed in hemoglobin concentration (Table I) and the same was found with respect to serum iron concentration and TIBC before and one year after the insertion of the IUD (Table II) However due to technical failure serum iron concentration and TIBC were determined only in 15 women but these women had a mean increase of the menstrual blood loss one year after insertion which amounted to ?6 1 ml corresponding to 78% of the control periods Thus the increase in slightly

Table III. *Menstrual blood loss (ml) before and after insertion of IUD (Cu 7) in four women with menorrhagia*

| Patient | Age | Parity | Control periods 1 | Control periods 2 | Periods after insertion 1 | Periods after insertion ? | Periods after insertion 3 | Periods after insertion 4 | Periods after insertion 5 |
|---|---|---|---|---|---|---|---|---|---|
| M E | ?7 | II | 160 4 | 134 5 | 101 ? | 123 1 | 111 2 | 2?1 4* | 111 1 |
| S J | 31 | III | 155 2 | 134 2 | 49 0 | 105 8 | 7? 6 | 11? 5 | 57 4 |
| K N | 5 | II | 14 9 | 151 4 | 169 4 | 160 3 | 11? ? | 16? 0 | 8? 9 |
| M T | 9 | I | 1 8 4 | 106 6 | 113 8 | | | | |

The patient had 3 tablets of tranexamic acid last day of the period

higher in these 15 individuals than the mean determined one year after the insertion of the IUD in the total series Therefore the result can be regarded as representative for the whole series Other authors (1 2) have reported a considerably higher frequency of anemia in women with plastic nonmetallic IUD Thus it may be concluded that the increase of the menstrual blood loss due to use of copper 7 IUD does not usually change the iron state However the risk for development of menorrhagia although small must still be kept in mind

The mechanism for the increase of the blood loss in women with IUD is not clear However there are some facts indicating that an increased fibrinolytic activity in the endometrium may be of importance Thus it has been shown (5 7 10) that administration of antifibrinolytic drugs are effective in reducing increased menstrual blood loss in women using IUD It has also been suggested (5 7) that the IUD may cause a traumatic effect of the endometrium resulting in an excessive release of plasminogen activators from necrotic cells Studies are in progress in order to evaluate this assumption In monkeys it has been shown that the fibrin proteolysis was higher in periods in which IUD was present in the uterus (9) Further investigations are needed to elucidate if the less pronounced increase of menstrual blood loss using the copper IUD are mainly due to the small size of the IUD and thus mechanical or if the copper in elf may be of specific importance

Four women with menorrhagia before insertion of UD were included in the present study It is remarkable that in general no increase of the blood loss occurred in these women It may be hazardous to draw any conclusions from these few cases but the trend is interesting Menorrhagia has been considered as a contraindication for use of IUD To judge from the present results this statement may be unreliable Further studies are however necessary in order to evaluate the effect of IUD on heavy menstrual blood losses One possible explanation for the fact that the blood loss does not increase to the same extent in menorrhagic women compared to women with normal menstrual blood loss may be that the endometrium in women with menorrhagia has such a high fibrinolytic activity that a further release of plasminogen activators due to IUD-trauma is negligible

## ACKNOWLEDGEMENTS

Our thanks are due to Mrs Sonia Forshell for devoted technical assistance This work was supported by grants from G D Searle Ltd USA from AB Kabi Sweden and from the Medical Research Council (B73-03X ?873-04) Sweden

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Submitted for publication March 4 1974

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Acta Obstet Gynecol Scand 54 319–323 1975

# INFLUENCE OF INTRAUTERINE HAEMOLYSIS ON THE AMNIOTIC FLUID PHOSPHOLIPID CONCENTRATION

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*Abstract* The relationship between surfactant synthesis measured by amniotic fluid lecithin concentration and L/S ratio and the degree of intrauterine haemolysis as judged by the Liley optical density difference has been studied in 125 samples from 67 rhesus immunized pregnant women In forty cases where the samples were obtained within one week of delivery the results have been correlated with cord blood haemoglobin concentrations There is evidence that a mild intrauterine haemolytic process may stimulate surfactant production while severe intrauterine haemolysis may be inhibitory in some cases Intrauterine anaemia did not seem to have any effect upon respiratory function after birth as long as the L/S ratio was adequate

In a previous paper Lindback et al (6) have reported some cases of erythroblastosis fetalis with low phospholipid concentrations in the amniotic fluid at 36–38 weeks gestation who subsequently developed respiratory distress syndrom (RDS) Whitfield et al (13) have noted a failure of the lecithin/sphingomyelin area ratio to rise in some pregnancies complicated by severe rhesus incompatibility Two cases of hydrops fetalis with delayed pulmonary maturation have also been reported by Gluck & Kulovich (4)

It seemed reasonable to assume that intrauterine anaemia could adversely affect surfactant synthesis in fetal lungs In the present study we have examined the influence of the degree of intrauterine haemolysis upon the amniotic fluid phospholipid concentrations during the last trimester of pregnancy

## MATERIAL AND METHODS

125 samples of amniotic fluid were obtained by amniocentesis of 67 rhesus immunized pregnant women 19 samples from eleven cases treated by intrauterine transfusion are not included because of uncertainty about interpretation of the phospholipid concentrations Five samples obtained early and late in pregnancy were also excluded being too few for data processing as was one pregnancy complicated by toxaemia The lecithin concentrations and L/S ratios for the remaining 100 of these samples were divided into three groups according to the Liley index Group I corresponds to Liley Zone I and group II includes the lower half of Zone II Group III corresponds to the upper half of Zone II plus Zone III according to the optical density difference The data from the individual groups have been compared statistically at two-weekly periods from 29 to 39 weeks gestation using the Wilcoxon two-sided two-sample test

Forty samples were obtained within one week of delivery In this group lecithin concentrations and L/S ratios have been correlated with haemoglobin concentrations in cord blood

The method of amniotic fluid centrifugation lipid extraction and quantitation as well as the assessment of respiratory function of the newborn have been reported previously (6) In the present study RDS was associated with lecithin concentration <1 3 micromoles in 100 ml of amniotic fluid and L/S ratios <2 0 These results are not comparable with other reports due to a difference in methodology (7 8)

## RESULTS

The results of the lecithin and L/S ratio analysis are presented in Table I The median values for each two-weekly gestational period and for each Liley group are shown in Figs 1 and 2 The lecithin concentration and L/S ratios are usually low until 34 weeks gestation when an increase in the L/S ratio becomes evident The lecithin concentrations showed no obvious rise until after 35 weeks gestation Thereafter the median values of both parameters show a marked increase in all groups

In Liley group III there were no samples after 37 5 weeks gestation Patients with such a severe intrauterine haemolysis were all delivered before that time

Table I *Lecithin concentrations in μM/100 ml amniotic fluid and lecithin/sphingomyelin (L/S) ratio grouped according to Liley chart at different gestational ages*

O D =Optical density difference

| Gestational age in weeks | Group I | | | Group II | | | Group III | | |
|---|---|---|---|---|---|---|---|---|---|
| | O D | Lec | L/S | O D | Lec | L/S | O D | Lec | L/S |
| 28–29 | | | | 0 059 | 0 57 | 1 14 | 0 166 | 0 7? | 1 05 |
| | | | | 0 07? | 0 97 | 1 21 | 0 ?01 | 0 76 | 1 41 |
| | | | | 0 104 | 1 03 | 1 28 | 0 193 | 1 78 | 1 49 |
| | | | | 0 090 | 1 10 | 1 50 | | | |
| Median | | | | | 1 00 | 1 25 | | 0 76 | 1 41 |
| 30–31 | | | | 0 077 | 0 91 | 1 00 | 0 105 | 0 54 | 1 20 |
| | | | | 0 080 | 0 95 | 1 07 | 0 ?16 | 0 95 | 1 50 |
| | | | | 0 076 | 1 27 | 1 25 | 0 127 | 1 20 | 1 62 |
| | | | | 0 082 | 1 68 | 1 58 | 0 121 | 1 86 | 1 90 |
| Median | | | | | 1 11 | 1 16 | | 1 08 | 1 56 |
| 32–33 | 0 036 | 0 17 | 0 90 | 0 056 | 0 62 | 1 15 | 0 100 | 0 3? | 0 98 |
| | 0 0 | 0 84 | 1 28 | 0 055 | 0 69 | 1 20 | 0 072 | 0 77 | 1 6? |
| | 0 035 | 0 85 | 2 00 | 0 077 | 0 81 | 1 64 | 0 118 | 0 96 | 1 71 |
| | 0 016 | 0 87 | 2 02 | 0 090 | 1 02 | 1 66 | 0 293 | 1 72 | 1 84 |
| | | | | 0 052 | 1 05 | 1 88 | 0 141 | 1 92 | 1 90 |
| | | | | 0 057 | 1 20 | 2 14 | | | |
| | | | | 0 081 | 1 39 | 2 35 | | | |
| | | | | 0 054 | 1 61 | 2 43 | | | |
| Median | | 0 85 | 1 64 | | 1 04 | 1 78 | | 0 95 | 1 71 |
| 34–35 | 0 034 | 0 18 | 0 90 | 0 065 | 0 48 | 1 00 | 0 093 | 0 30 | 0 77 |
| | 0 0 6 | 0 98 | 2 12 | 0 040 | 0 89 | 1 39 | 0 126 | 0 34 | 1 07 |
| | 0 0?9 | 1 19 | ? 22 | 0 052 | 1 13 | 1 90 | 0 092 | 0 37 | 1 08 |
| | 0 0?8 | 1 23 | 2 56 | 0 057 | 1 20 | 1 91 | 0 103 | 0 48 | 1 28 |
| | | | | 0 06? | 1 54 | 2 44 | 0 109 | 0 51 | 1 30 |
| | | | | 0 060 | 1 58 | 3 20 | 0 085 | 0 90 | 1 59 |
| | | | | 0 070 | 1 86 | 3 22 | 0 120 | 0 94 | 1 74 |
| | | | | 0 039 | 1 96 | 3 40 | 0 337 | 3 74 | 4 90 |
| | | | | 0 070 | 2 00 | 3 60 | | | |
| | | | | 0 063 | 2 11 | 5 04 | | | |
| Median | | 1 09 | 2 17 | | 1 56 | 2 82 | | 0 50 | 1 9 |
| 36–37 | 0 015 | 0 22 | 1 00 | 0 070 | 0 46 | 0 79 | 0 129 | 0 68 | 1 13 |
| | 0 017 | 1 10 | 1 80 | 0 048 | 0 57 | 2 38 | 0 074 | 0 89 | 1 23 |
| | 0 0?0 | 1 29 | 2 39 | 0 037 | 1 18 | 3 46 | 0 080 | 1 47 | 64 |
| | 0 0_0 | 1 45 | 3 38 | 0 038 | 1 19 | 4 20 | 0 080 | 1 51 | 2 9? |
| | 0 015 | 1 66 | 3 82 | 0 043 | 1 4? | 4 21 | 0 103 | 2 03 | 3 75 |
| | 0 014 | 1 67 | 4 18 | 0 044 | 1 57 | 4 58 | 0 118 | 2 45 | 4 2 |
| | 0 0?7 | 2 38 | 5 36 | 0 072 | 1 77 | 4 90 | 0 090 | 3 04 | 5 20 |
| | 0 017 | 2 74 | 6 80 | 0 042 | 2 86 | 5 10 | | | |
| | | | | 0 055 | 2 93 | 5 64 | | | |
| | | | | 0 056 | 3 24 | 6 55 | | | |
| | | | | 0 057 | 4 23 | 11 06 | | | |
| Median | | 1 56 | 3 60 | | 1 57 | 4 58 | | 1 51 | ? 9? |
| 38–39 | 0 0?0 | 0 33 | 1 75 | 0 026 | 0 77 | 2 16 | | | |
| | 0 0?0 | 0 74 | 1 83 | 0 050 | 1 26 | 2 72 | | | |
| | 0 019 | 1 46 | 2 33 | 0 024 | 1 44 | 4 74 | | | |
| | 0 0 | 1 72 | 2 92 | 0 045 | 2 37 | 4 80 | | | |
| | 0 016 | 2 19 | 5 76 | 0 030 | 2 99 | 6 44 | | | |
| | 0 005 | 2 38 | 5 80 | 0 025 | 3 68 | 6 77 | | | |
| | 0 020 | 3 72 | 7 16 | 0 048 | 4 25 | 8 43 | | | |
| | 0 018 | 5 05 | 7 20 | 0 042 | 4 76 | 10 00 | | | |
| | 0 0?7 | 5 18 | 7 50 | 0 031 | 5 49 | 13 1 | | | |
| | 0 003 | 5 34 | 10 90 | | | | | | |
| | 0 0 | 5 64 | 11 30 | | | | | | |
| Median | | 2 38 | 5 80 | | 2 98 | 6 74 | | | |

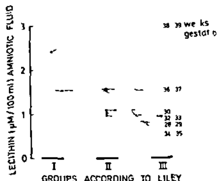



*Fig 1* Relationship between the median lecithin concentrations for each Liley group (see text) ranged according to gestational age

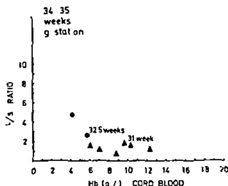



*Fig 3* Relationship between the L/S ratios obtained within one week of delivery and cord blood haemoglobin concentration ● Normal respiration ▲ RDS

It appears from Figs 1 and 2 that the median values of lecithin and L/S ratio are highest in group II which include patients judged to have a mild degree of intrauterine haemolysis The lecithin concentration and the L/S ratio in the severely haemolytic group (III) are markedly lower than in group II and also lower than in group I which includes patients with no pathological intrauterine haemolysis Using the Wilcoxon two-sample test on the data presented in Table I we have found that the difference in L/S ratios between groups II and III at 34–35 weeks gestation is statistically significant $\alpha=4\ 6\%$ At 36–37 weeks the difference is not statistically significant $\alpha=14\ 8\%$ Combining the data for 34–35 and 36–37 weeks and using the Wilcoxon–van Eltern test does however give a statistically significant difference between groups II and III $\alpha=1\ 2\%$

The lecithin levels at 34–35 weeks gestation show the same variations but the measurements at 36–37 weeks demonstrate no difference among the groups The difference seen at 34–35 weeks between groups II and III is statistically significant with the Wilcoxon two sample test $\alpha=3\ 4\%$

The differences between groups I and II for lecithin and L/S ratio are not statistically significant and neither are the differences between groups I and III

Figs 3 4 and 5 show the relationship between the L/S ratio obtained within one week of delivery and cord blood haemoglobin in 40 newborn infants Eight infants delivered prior to 36 weeks gestation (Fig 3) were all anaemic The L/S ratio was <2 0 in six cases and all developed RDS The one with the highest L/S ratio had the lowest haemoglobin concentration

15 patients were delivered at 36–37 weeks gestation (Fig 4) Five infants had cord haemoglobin concentrations <10 g% Their median L/S ratio is somewhat lower than for those who had higher haemoglobin concentrations but there is no obvious

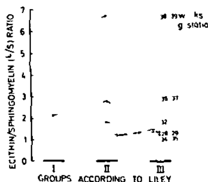



*Fig 2* Relationship between the median L/S ratios for each Liley group (see text) ranged according to gestational age

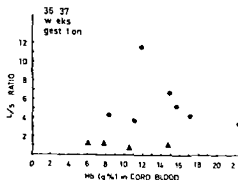



*Fig 4* Relationship between the L/S ratios obtained within one week of delivery and cord blood haemoglobin concentration ● Normal respiration ▲ RDS

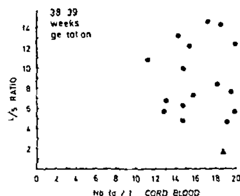



*Fig 5* Relationship between the L/S ratios obtained within one week of delivery and cord blood haemoglobin concentration ● Normal respiration ▲ RDS

trend or statistically significant difference The L/S ratio was <1 5 in the four cases who developed RDS Three of these died

Of the 17 patients delivered after 38 weeks gestation (Fig 5) none were severely anaemic Seven had haemoglobin concentrations from 10–15 g% There is no difference in the L/S ratio among these patients and the rest of the group who had haemoglobin concentrations >15 g% The lowest L/S ratio in this group was associated with one of the highest haemoglobin concentrations

## DISCUSSION

Reviewing the results of the present study we find a tendency to higher lecithin concentrations and L/S ratios in cases with mild intrauterine haemolysis while a severe haemolytic process seems to result in reduced surfactant production in some cases Compared with the group of infants judged to have no pathological haemolysis neither the tendency to increased nor reduced surfactant production in the two groups mentioned above are statistically significant The finding of a statistically significant difference between the groups with mild and severe intrauterine haemolysis indicate however a real tendency The accelerated development of the surfactant system in cases with a mild haemolytic process probably indicates that this is sufficiently stressful to activate the enzymatic system responsible for lecithin synthesis Naeye et al (11) and Bauer et al (1) have found that antenatal bacterial infections and prolonged rupture of membranes offer some protection against the development of RDS Infections and other forms of stress have been shown to stimulate corticosteroid production and induce hypertrophy of the adrenal cortical adult zone cells (3 9 10) Liggins & Howie (5) have reported a decreased incidence of RDS in prematurely delivered infants after corticosteroid infusions to the mother The accelerated erythropoiesis and other adjustments to mild heamolysis in our patients may therefore similarly activate the adrenal cortex

The tendency to low lecithin concentrations and L/S ratios in severely anaemic infants suggests that anaemia poor perfusion hypoxia and acidosis may impair the synthesis of lecithin in the lungs either directly or through reduced adrenal cortical function This is however not a constant finding since even a highly anaemic child may have normal respiratory function after birth It is tempting to speculate whether the duration of the anaemia is the significant factor The consequences to the fetus of severe intrauterine anaemia is probably similar to that of asphyxia Both in the human infant (2) and in the lamb (12) it appears that asphyxia enhances the risk of the development of hyaline membrane disease

The comparison between the cord blood haemoglobin concentration and the L/S ratio in samples obtained within one week of delivery is inconclusive Infants delivered prior to 36 weeks gestation were all severely anaemic except one On the other hand at 38–39 weeks none were severely anaemic For these groups therefore an evaluation of the influence of the degree of anaemia upon the surfactant production yields very little information The fact that 6 of 8 infants delivered before 36 weeks gestation had an L/S ratio less than 2 0 and developed RDS which is a higher frequency than would be expected at this gestational age probably indicates that some inhibition of surfactant production has taken place

In infants delivered at 36–37 weeks gestation there is no convincing trend in the comparison between the haemoglobin concentration and the L/S ratio even though the median L/S ratio is somewhat lower for the severely anaemic group than the rest

In our study 11 infants developed RDS and all had low L/S ratios Even though 7 of these infants were severely anaemic anaemia by itself did not seem to affect respiration in infants with an adequate L/S ratio

Our findings do not permit definite conclusions to be drawn There is support for the view that mild intrauterine heamolysis may stimulate or accelerate

lecithin production while severe intrauterine haemolysis may inhibit lecithin production in some cases The degree of anaemia by itself does not seem to contribute to the development of the respiratory distress syndrome other than through its influence upon surfactant production


## ACKNOWLEDGEMENTS

We are indebted to Prof K Bjøro Department of Obstetrics and Gynaecology Prof M Seip Department of Paediatrics and Norske Kvinners Sanitetsforening for making this study possible We also wish to thank Associate Professor Lars Walløe Departments of Physics and Physiology University of Oslo for his assistance with the statistical analysis


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*Submitted for publication March 12 1974*

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Acta Obstet Gynecol Scand 54 325–328 1975

# QUANTITATIVE ANALYSIS OF RADIOISOTOPIC ANGIOGRAPHY IN TROPHOBLASTIC NEOPLASIA

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*Abstract* The diagnostic significance of radioisotopic angiography in the clinical management of trophoblastic neoplasia was studied by analyzing the patterns of radioisotope (RI)-dynamic curve obtained by processing sequential image with a computer and an attempt was made to express numerically the size of tumors present in vivo It was demonstrated that the total activities of RI in the abnormal phase of RI-dynamic curves in trophoblastic neoplasia were closely correlated with the sizes of the intrauterine tumors actually resected

Recently pelvic angiography has been frequently employed for the clinical management of trophoblastic neoplasia

Pelvic angiography being useful in diagnosing the site size and mode of spread of tumor is often used for assessing the effects of chemotherapy with anti cancer agents (4) as well as one of the presurgical diagnostic examinations (1)

It is not easy however to analyze qualitatively and quantitatively the characteristics of trophoblastic neoplasia by referring only to pelvic angiograms

An attempt was therefore made by the authors (5) to analyze the clinical characteristics of trophoblastic neoplasia with the method of radioisotopic angiography and processing with a computer the sequential images of radioisotopic activity in the tumor

## METHOD OF ANGIOSCINTIGRAPHY

The equipment used by the authors is the scinticamera of the videotape recorder on line system equipped with a mini-computer (the Toshiba nuclear medical data processing system Fig 1)

8 mCi/10 ml of $^{99m}$Tc pertechnetate is infused by following Seldinger s method into the abdominal aorta via a catheter inserted through the femoral artery and the distribution of the radioisotope inside the pelvic cavity is recorded with the detector of the scinticamera which is equipped with a 4 000 hole collimeter under the conditions of energy 140 KeV and C W 40%

The analogue images of x–y coordinates appear on the cathode ray tube (CRT) at this time and these are converted into the positional signs in the 128 × 128 matrix via an analogue to-digital converter and recorded in real time on a videotape

When the videotape is played back digital images are displayed If the area in the image which represents a tumor is divided up and if the radioactive transitions in these divisions are processed in the computer information is then available of the radioactivity in the tumor

## FINDINGS OF RADIOISOTOPIC ANGIOGRAPHY

### 1 *Sequential images*

Shown in Fig 2a b and c are the sequential images of the radioactivity displayed on the CRT of scinticamera photographed with a polaroid camera at 1 5 sec intervals and compared with the pelvic angiograms synchronized with the images

The RI images representing the iliac artery uterine artery and uterine hypervascularity are obtained at 3 sec after the infusion of $^{99m}$Tc and the RI image corresponding to the tumor stain on pelvic angiogram at 4 5 sec after the infusion The return of radioisotope to the venous system is seen at 7 5 sec after infusion of $^{99m}$Tc and it is obvious that the radioisotope image at this stage corresponds to the pooling shadow in the pelvic angiogram

### 2 *Abnormal RI dynamic curve*

The playback of the video tape displays (the digital images as shown in Fig 3) on the CRT If the area in

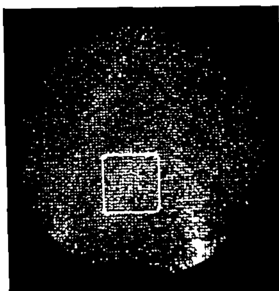


*Fig 3* Digital RI image and the split area assigned to abnormal region

of the tumor site measured with a planimeter and the values used as the measure of tumor size

## DISCUSSION

The scintillation camera detects the intravisceral dynamics of the RI infused into the living organism as sequential RI images and the use of the computerized image processing system in combination

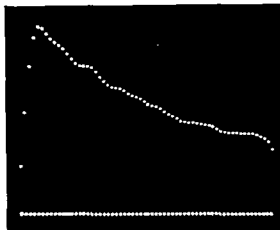


*Fig 5* RI dynamic curve in split area of normal pregnancy

with the camera permits the evaluation of the dynamic images of RI as digital data (3)

Trophoblastic neoplasia lacks stroma and possesses such a characteristic architecture that tumor cells are suspended in a blood lake For this reason the hot image due to accumulation of RI in tumor is displayed in radioisotopic angiography and variations in the image with time from its appearance till disappearance are synchronized with the findings of pelvic angiography (2)

The analysis of RI dynamics in this hot image demonstrates abnormal hemodynamics in the tumor site It is therefore possible to assess the architecture of the tumor by recognizing the pattern of the diminishing portion of the RI dynamic curve

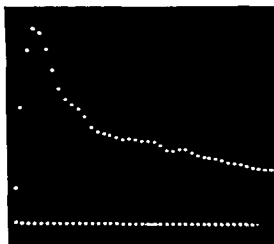


*Fig 4* RI dynamic curve in split area of trophoblastic neoplasia.

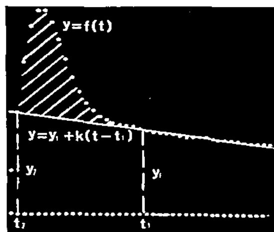



*Fig 6* Mathematical model designed for the RI total count in abnormal phase of RI dynamics curve

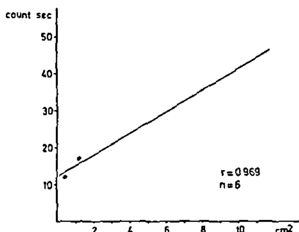



*Fig 7* Correlation of RI total count in abnormal phase and size of tumor

For example the presence of an arteriovenous shunt produced by the tumor can be indicated by a rapidly decreasing tendency in the diminishing portion of the RI dynamic curve This decrease ought to be slow with a more normal blood supply In reality the diminishing portion of the RI dynamic curve often comprises three components including the terminal phase

It has been demonstrated by the authors (6) that when there is connective tissue formed around a tumor as a result of chemotherapy there is a gradual slope of the diminishing portion of the RI dynamic curve Thus the pattern of the RI dynamic curve permits an evaluation of the architecture of the tumor

The numerical expression of the size of a tumor in situ is necessary for selecting the therapy and evaluating the efficacy of chemotherapy For this *purpose the total RI count in the abnormal phase of* the RI dynamic curve was determined as the count of RI activity that flowed into the tumor site and by comparing the findings with the sizes of intrauterine tumors that were resected a correlation was noted between them It is strongly recommended that this method is used for measuring the dynamic of a tumor which is not easy to assess by pelvic angiography

Because it is feasible with the computerized scintillation camera system to record on videotape all the information obtained about a tumor it is possible by the addition of data processing to extract the necessary information about the tumor which will contribute to accurate diagnosis and the authors believe that this technological progress will contribute to the diagnosis of trophoblastic neoplasia

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*Submitted for publication March 18 1974*

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*Fig 8* Gross section of excised uterus including trophoblastic neoplasia

Acta Obstet Gynecol Scand 54 329–332 1975

# THE RELATIONSHIP BETWEEN THE CEPHALO-PEDAL PROGRESS OF CLINICAL ICTERUS AND THE SERUM BILIRUBIN CONCENTRATION IN NEWBORN INFANTS WITHOUT BLOOD TYPE SENSITIZATION

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*Abstract* The correlation between the cephalo pedal progress of clinical icterus and the serum bilirubin concentration was examined in newborn infants without blood type sensitization In daylight 374 observations were made 290 on 171 mature infants and 84 on 24 premature infants The investigation showed that a caudad progress of icterus corresponded with an increasing serum bilirubin concentration within certain intervals The observation of how far icterus had progressed can thus be helpful in the clinical evaluation of the serum bilirubin concentration and repeated observations can demonstrate whether icterus progresses showing that the concentration is increasing The same relationship was found in respect of all the infants with the exception of the two smallest (birth weight ≤1 000 g) They were icteric on the feet at lower concentrations than the other infants Furthermore the relationship did not depend on which day the observations were made It was not necessary to determine the bilirubin concentration until icterus had progressed to below the knees as a concentration ≤110 mg/l corresponded to all observations in which the lower limit of icterus was found proximal to the knees However in the small premature infants it would have been reasonable to measure the concentration when icterus had reached the area below umbilicus

Careful observation of newborn infants is necessary in order to prevent encephalopathy caused by unconjugated bilirubin Various investigations have been made of the correlation between the intensity of clinical icterus and the serum bilirubin concentration and a rough connection has been found (2 3 4 6) Porak (11) reported in 1878 that icterus in newborn infants is cephalad in the start and caudad in progression he divided icterus into three degrees In the 1st degree the face breast and back were coloured in the 2nd degree also abdomen upper arms and thighs and in the 3rd degree icterus had become universal In the present study the connection between the progress of icterus and the serum bilirubin concentration was investigated in newborn infants without blood type sensitization A corresponding study was made in 1969 by Kramer (8) but the observations of icterus took place in blue white light the author finding the variations in the volume of daylight too large for the purpose and the variation in serum bilirubin concentration corresponding to a certain lower limit of icterus was large In the present study the observations were performed in daylight partly in order that the results might be applicable in the general clinical situation—icterus being normally observed in daylight—partly because preliminary work proved that the spreading in serum bilirubin concentration at a certain lower limit of icterus was less when icterus was observed in daylight than in blue white light Furthermore a study was made of whether the relationship between the extension of icterus and the serum bilirubin concentration depended on how many days the infants were old when the observations were carried out

## OWN INVESTIGATIONS

### *Material and Methods*

The investigation was carried out between the 1st March and the 1st June 1973 and comprised newborn icteric infants without blood type sensitization None of the infants received phototherapy They were examined completely naked once daily in much varied daylight The colour of the skin was evaluated after having faded by pressure with the thumb and the lower limit of icterus was determined The skin surface was arbitrarily divided into five regions (1) head and neck (2) trunk to the umbilicus (3) the lower part of the body and the lower extremities to the knees (4) the lower extremities from

Table I *Two individual examples of the relationship between the progress of clinical icterus and the serum bilirubin concentration*

| | Region | Serum bilirubin concentration (mg/l) |
|---|---|---|
| 1 day old | Non icteric | Not determined |
| 2 days old | ? | 61 |
| 3 days old | 3 | 109 |
| 4 days old | 4 | 178 |
| 5 days old | 5 | 143 |
| 6 days old | 5 | 166 |
| 1 day old | Non icteric | Not determined |
| 2 days old | 1 | 47 |
| 3 days old | 2 | 53 |
| 4 days old | 2 | 60 |
| 5 days old | 2 | 66 |

knees to ankles and (5) feet The arms were not included since the preliminary work had shown that there was no fixed correlation between the progress of icterus on the upper and lower extremities After examination of the infants the serum bilirubin concentration was determined on capillary blood by diazotization in basic liquid by a modification of Jendrassik & Gróf's method (9) When the serum bilirubin concentration started to fall the infants were excluded from the investigation since the preliminary work had shown that once the concentration started to fall icterus would fade gradually in all affected areas at the same time rather than in the cephalad direction Thus the investigation included infants with increasing and possibly stationary hyperbilirubinemia and they were not included after the age of 8 days A total of 374 observations were made 290 on 121 mature infants and 84 on 24 premature infants (birth weight ≤2 500 g) All observations were made by the same person

## RESULTS

Table I shows two individual examples of the relationship between the progress of icterus and the serum bilirubin concentration The infants became icteric at the concentrations ≥34 mg/l first on the head and neck and some of the infants simultaneously on the upper part of the body From here the progress of icterus was caudad with increasing hyperbilirubinemia to include eventually the feet as well In stationary hyperbilirubinemia the lower limit of icterus remained unchanged

The relationship between the extension of icterus and the serum bilirubin concentration for the total number of infants is shown in Fig 1 and Table II It appears that premature infants did not differ

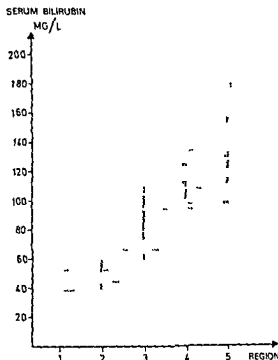



*Fig 1* The relationship between the extension of clinical icterus and the serum bilirubin concentration in premature (×) and mature ( ) infants without blood type sensitization The four lowest observations in region 5 were made in the two smallest infants of the material with birth weight ≤1 000 g

from the mature infants with the exception of the two smallest ones who were icteric in the feet at lower bilirubin values than the other infants When icterus was localized to head and neck (region 1) the concentration was 34–69 mg/l (on an average

Table II *The relationship between the extension of clinical icterus and the serum bilirubin concentration in new born infants without blood type sensitization*

| | Serum bilirubin concentration mg/l | | Number of observations |
|---|---|---|---|
| | Range | Average ±S D | |
| Region 1 | 34–69 | 47±8 | 33 |
| Region 2 | 37–84 | 58±1? | 6? |
| Region 3 | 57–110 | 84±13 | 121 |
| Region 4 | 87–166 | 113±17 | 85 |
| Region 5 all infants | ≥93 | 144±?? | 68 |
| infants with birth weight >1 000 g | ≥112 | 147±19 | 64 |

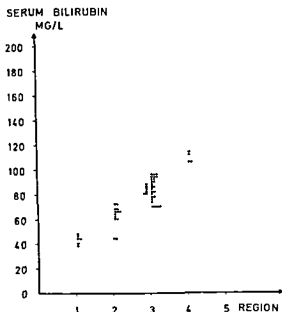



*Fig* ? The relationship between the extension of clinical icterus and the serum bilirubin concentration on the third day of life in premature (×) and mature ( ) infants without blood type sensitization The two lowest observations in region 5 were made in the two smallest infants of the material with birth weight ≤1 000 g

47 mg/l) If also the upper part of the trunk (region 2) was coloured 37–84 mg/l (on an average 58 mg/l) If icterus had progressed to the lower part of the body and to the thighs (region 3) the concentration was 57–110 mg/l (on an average 84 mg/l) to the lower part of the lower extremities to the ankles (region 4) 87–166 mg/l (on average 113 mg/l) and to the feet (region 5) ≥112 mg/l The two smallest infants with birth weight ≤1 000 g were however icteric in the feet at a concentration of 93–99 mg/l Of course comparison between the bilirubin values of the adjacent skin region showed much overlap but comparison between regions 1 3 and 5 showed slight overlap

By means of the Mann Whitney test the average serum bilirubin concentrations of adjacent regions were compared The difference was significant (region 1 as compared with region 2 $P<0.01$ in the other cases $P$ much less than 0 001) In order to avoid dependence in the observations the test was performed in a reduced series containing only one observation per infant and this was selected by random

The relationship between the extension of icterus and the serum bilirubin concentration was examined on each day (as an example the third day of life is illustrated in Fig 2) and for all days the concentration corresponding to a certain skin region was found to be within the same interval Thus the relationship appeared not to depend on how many days the infants were old when the observations were carried out

## DISCUSSION

The investigation showed that to caudad progress of clinical icterus in newborn infants without blood type sensitization corresponded with an increasing serum bilirubin concentration within certain intervals The observation of how far icterus has proceeded may thus be of help in the clinical evaluation of the serum bilirubin concentration and repeated observations can prove whether icterus is progressing showing that the concentration is increasing The relationship between the progress of icterus and the serum bilirubin concentration was found to be the same for premature and mature infants with the exception of the quite small premature infants who were icteric in the feet at lower concentrations than the other infants The relationship did not depend on which day the observations were carried out The variation in serum bilirubin concentration at a certain lower limit of icterus was considerably less than stated by Kramer (8) The reason partly was that the observations of icterus were carried out in daylight partly that the progress of icterus on the upper extremities was not included in the study as was the case in Kramer s study the preliminary work having shown that there was no fixed relationship between the progress of icterus on the upper and lower extremities as also previously pointed out by Porak (11) No explanation is available of the progress of icterus

My own investigation comprised infants with slight and moderate degrees of hyperbilirubinemia Recognition of the moderate degrees is desirable as they may involve risk of brain damage Boggs et al (1) thus found by examining 8 months old children—mature and premature—a positive relationship between increasing neonatal hyperbilirubinemia and reduced motor and/or mental function which was significant at a concentration of 160 mg/l Furthermore Plum et al (10) thought it probable that children with a history of neo-

natal icterus without blood type sensitization had slower motor development during the first year of life than the non icteric children (if necessary the children had received replacement transfusion) and small premature infants without hemolytic disease developed kernicterus although the concentration had not exceeded 110–180 mg/l (5 7 12)

In my own investigation including infants with out hemolytic disease it was not necessary to measure the serum bilirubin concentration until icterus had progressed as far as below the knees in view of the fact that a concentration ≤110 mg/l (Fig 1 and Table I) was found in all 221 observa tions in which the lower limit of icterus was found proximal to the knees (including region 3) How ever in the small premature infants it would have been reasonable to measure the concentration when icterus had reached the area below the um bilicus The evaluation of the lower limit of icterus will be somewhat individual but the examiner will soon obtain experience in how to investigate the serum bilirubin concentration

## ACKNOWLEDGEMENT

The statistical work in this investigation was performed by Aage Nielsen M Sc Aalborg

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*Submitted for publication March 27 1974*

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Acta Obstet Gynecol Scand 54 333–340 1975

# TREATMENT OF PRURITUS IN CHOLESTASIS OF PREGNANCY WITH A NEW ANION EXCHANGE RESIN (SECHOLEX®)

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*Abstract* Anion exchange resins form a non-absorbable complex with bile acids in the intestine thus removing bile acids from the enterohepatic circulation and facilitating bile acid excretion in the faeces A new bile acid sequestrant (PDX chloride Secholex®) was evaluated for the relief of pruritus in cholestasis of pregnancy (CP) in 31 women CP was verified by the presence of the abnormal lipoprotein X in serum and the clinical series was divided into two degrees of severity pruritus gravidarum (PG) and hepatosis of pregnancy (HP) based on liver function tests Eleven of 31 women discontinued treatment because of gastro-intestinal side effects Of the 20 women continuing the study for more than one week all with a milder form of cholestasis PG ($n=8$) experienced relief of pruritus while some relief was obtained in 75% of the women with HP After up to 4 weeks administration of Secholex no obvious interference with fat absorption was evident judging from the serum lecithin content of linoleic and arachidonic acids A reduction in serum folic acid might indicate an interaction in folic acid absorption An expected reduction in serum cholesterol levels which are characteristically increased in CP was not achieved by the administration of Secholex

Jaundice of pregnancy with a recurrent tendency in subsequent pregnancies was first described by Ahlfeldt (cf 12) and was recognized as a clinical entity twenty years ago by Svanborg et al (21 22) and Thorling (26) Pruritus gravidarum is a milder form of this complication in which morphological examination of the liver has revealed changes similar to those seen in cholestasis (1)

Pruritus is often the initial and dominating symptom in cholestasis of pregnancy (CP) occasionally followed by jaundice and a concomitant increase in serum transaminases and alkaline phosphatase (12 13 26)

Cholestatic conditions in general are known to cause characteristic alterations in serum lipids i e elevated free cholesterol and phospholipids as well as in serum lipoproteins e g the occurrence of the abnormal serum lipoprotein X (LP X) (19)

In cholestatic conditions increased amounts of bile acids as well as other constituents of bile accumulate in the blood and in the skin (18) Bile acids in the skin are known to cause pruritus in cholestatic conditions Different means of removing bile acids from the enterohepatic circulation have been tried in cholestatic conditions of different origin Anion exchange resins are known to form a non absorbable complex with bile acids in the intestine thus removing the latter from the entero-hepatic circulation and facilitating their excretion in the faeces (25) Such resins have been shown to significantly reduce serum bile acid concentrations (5) and simultaneously to relief pruritus (5 8 11 17) Anion exchange resins suitable for oral administration have been available for more than 10 years (3 5) Among these bile acid sequestrants the new poly 2(diethylamino)ethyl) polyglycerylene dextran hydrochloride PDX chloride Secholex® appears to be most promising (9)

The aim of the present study was primarily to investigate the effect of orally administered Secholex on pruritus in women with CP Secondarily we wanted to elucidate the influence of Secholex on liver function tests and hematological data as well as its effect on serum lipids and lipoproteins including the fatty acid composition of serum lecithin

## MATERIALS AND METHODS

*Cl n cal series*

Thirty-one women with pruritus during pregnancy attending a pruritus out patient clinic at the Maternal

Table I *Clinical data in 20 women with cholestasis of pregnancy (pruritus gravidarum case number 1–8 and hepatosis of pregnancy case number 9–20) during oral treatment with Secholex® (5 g t i d )*
N D =Normal delivery V E =vacuum extraction

| Case no | Para | Onset gest week | Treat ment started gest week | Dura tion of treat ment (weeks) | Deliv ery week | Delivery | Remarks | Weight of pla centa (g) | Birth weight (g) | Infant diagnosis |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | I | 33 | 33 | ? | 35 | N D | | 480 | 2 040 | Immaturity |
| 2 | IV | 37 | 37 | 3 | 40 | N D | | 650 | 3 360 | Normal |
| 3 | I | 32 | 35 | 4 | 39 | Caesarean section | Narrow pelvis | 640 | 3 800 | Normal |
| 4 | 0 | 20 | 39 | 1 | 41 | N D | | 7?0 | 3 610 | Hydrocele testis |
| 5 | 0 | ?6 | 34 | 5 | 39 | N D | Breech presentation | 500 | 3 300 | Hyperbil irubemia |
| 6 | II | 25 | 27 | 4 | 34 | N D | Cervical incompetence | 5?0 | 1 900 | Immaturity |
| 7 | II | 20 | 30 | 8 | 4? | N D | | 475 | 3 7?0 | Normal |
| 8 | 0 | 22 | ?4 | 3 | 4? | N D | Breech presentation | 780 | 3 140 | Normal |
| 9 | 0 | ?4 | 33 | 4 | 39 | V E | Asphyxia | 500 | 2 950 | Normal |
| 10 | 0 | 36 | 37 | 3 | 40 | N D | | 670 | 3 630 | Normal |
| 11 | I | 36 | 37 | ? | 39 | N D | | 700 | 4 000 | Normal |
| 12 | I | 32 | 33 | 4 | 39 | N D | | 530 | 3 110 | Normal |
| 13 | III | 30 | 34 | 4 | 39 | Caesarean section | Asphyxia | 700 | 4 200 | Normal |
| 14 | 0 | 33 | 37 | 2 | 39 | N D | | 530 | 3 600 | Normal |
| 15 | 0 | 36 | 37 | ? | 39 | N D | Duplex | 1 050 | 2 800 / ?610 | Normal |
| 16 | I | ?8 | 32 | 2 | 39 | N D | | 590 | 2 640 | Normal |
| 17 | 0 | 3? | 35 | 3 | 43 | N D | | 380 | 3 320 | Postmaturity |
| 18 | I | 34 | 36 | 3 | 38 | N D | | 530 | 3 400 | Normal |
| | 0 | ?? | ?9 | 4 | 38 | N D | | 540 | ? 3?0 | Light for date |
| | 0 | 33 | 37 | 5 | 43 | Caesarean section | Ablatio plac | 640 | 4 060 | Postmaturity |

Welfare Unit were selected for the trial These women all presented a history of generalized pruritus beginning during pregnancy and all were demonstrated to have the abnormal lipoprotein LP X in their sera These patients with cholestasis of pregnancy (CP) were divided into two clinical entities pruritus gravidarum (PG $n=14$) and hepatosis of pregnancy (HP $n=17$) depending on whether their serum bilirubin was below or above 1 2 mg/100 ml respectively and/or SGOT or SGPT below or above 50 Units/l respectively (15) The diagnosis of CP was further supported by the disappearance of pruritus after delivery in all cases

Of these 31 women 11 discontinued their treatment because of side effects The remaining twenty women with CP 8 with PG and 1? with HP (Table I) were studied for relief of pruritus

*Control values* for serum lecithin fatty acid composition were obtained from twenty non treated pregnant women randomly selected from the Maternal Welfare Unit without pruritus or any other condition complicating the pregnancy

*Secholex* (PDX chloride Pharmacia AB Uppsala Sweden) was composed of 5 g PDX chloride and 1 5 g gum acacia It was supplied in granular form and sealed in individual packages The dose was one package t i d taken orally with the meal or within 30 minutes after the meal The granules were mixed with water or fruit juice and allowed to stand for a couple of minutes before being taken The treatment was usually started 3–4 weeks after the onset of pruritus It was instituted in the 24th–40th gestational week (mean 34 3) and was continued for 1–8 weeks (mean 3 4 weeks)

A multivitamin preparation (Pancebrin®) was given parenterally (? ml) once weekly to each patient on Secholex therapy The preparation contained vitamin A ?0000 IE $B_1$ ?0 mg $B_2$ 4 mg $B_6$ 6 mg nicotinamide 40 mg panthothenic acid 6 mg ascorbic acid 1 0 mg D ?000 IE and E 4 mg

*Blood samples* were drawn in the fasting state in the morning at the time of the clinical examination The blood was allowed to clot for 30 minutes at room temperature and was then centrifuged at 2 500 g for 10 minutes after which the serum was immediately removed The serum specimens for gas liquid chromato-

Table II *Side effects in 31 patients with cholestasis of pregnancy (pruritus gravidarum PG n=14 and hepatosis of pregnancy HP n=17) after oral treatment with Secholex® (5 g t i d ) Number of cases*

| | Side effects | | | |
|---|---|---|---|---|
| | No side effects | Slight nausea constipation or diarrhoea | More pronounced nausea constipation or diarrhoea Discontinued treatment | Impossible to swallow or objectionable taste Discontinued treatment |
| PG n=14 | 5 | 3 | 3 | 3 |
| HP n=17 | 7 | 5 | 3 | 2 |

graphy (GLC) analyses were immediately frozen and stored at −20°C in glass tubes with teflon screw caps

*Liver function tests* Total serum bilirubin (normal <2 mg/100 ml) alkaline phosphatase (normal <8 Buch Units) SGOT (ASAT ) (normal <17 Units/l) and SGPT (ALAT[1]) (normal <17 Units/l) were determined at the Laboratory of Clinical Chemistry according to standard methods

*Lipids and lipoproteins* Serum triglycerides were determined according to Carlson (6) total cholesterol by the method of Cramér & Isaksson (7) and phospholipids as described by Svanborg & Svennerholm (23) The cholesterol content (16) of high-density lipoproteins (HDL) was quantificated after the precipitation of very low density lipoproteins (VLDL) and low density lipoproteins (LDL) with manganese chloride and heparin (4) The semi quantification of LP X was performed as described earlier (14) by a modified immuno-diffusion technique

*Hematological variables* Serum iron serum total iron binding capacity (TIBC) and Simplastin A were determined according to standard methods Folic acid in whole blood and serum was determined according to Hansen (10) at the Laboratory of Clinical Chemistry

*Gas liquid chromatography* Preparation of lipid extracts separation of lipids by thin layer chromatography on Silica gel and isolation of lecithin and phosphoglyceride spots and preparation of fatty acid methyl esters were performed as described earlier (13) Gas liquid chromatography of methyl esters measurement of serum lecithin and lecithin fatty acids and the conversion from weight per cent to mole per cent were described in an earlier publication (13) Apart from the fatty acids presented in the table 14 0 15 0 18 3 (n-6) 18 3 (n 3) 20 1 (n 9) 20 3 (n 9) 22 4 (n 6) 22 5 (n-6) and 22 5 (n 3) were identified but not included in the tables as their individual concentrations were generally less than 1%

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[1] *ASAT* (serum aspartate aminotransferase activity) and *ALAT* (serum alanine aminotransferase activity) in the new terminology are synonymous for *SGOT and SGPT* respectively In this publication the latter terms will be used

*Statistical methods*

Conventional methods were used for the calculation of means standard deviations and standard errors of means Student s *t* test was used to study differences between groups Values of $p \leq 0.05$ were considered statistically significant

## RESULTS

*Side effects* (Table II) Because of gastro-intestinal side effects treatment was discontinued in 11 (6 with PG and 5 with HP) of the 31 women with CP initially treated In five women withdrawal was due to objectionable taste and in six women due to nausea constipation and/or diarrhoea Among gastro-intestinal difficulties constipation was most frequent while nausea usually disappeared within the first week of Secholex administration

*Subjective relief of pruritus by Secholex* (Table III) In the twenty women continuing the treatment with Secholex for more than one week some relief of pruritus was noted in 16 of the women (80%) 7 of whom (38%) reported good relief Relief was

Table III *Subjective relief of pruritus in 20 women with cholestasis of pregnancy (pruritus gravidarum PG n=8 and hepatosis of pregnancy HP n=12) after oral administration with Secholex® (5 g t i d ) Number of cases*

| | | Subjective relief | | | |
|---|---|---|---|---|---|
| | n | None | Some | Moderate | Good |
| PG | 8 | 0 | 1 | 1 | 6 |
| HP | 12 | 4 | 3 | 4 | 1 |

Table IV *Effect on liver function tests during oral treatment with Secholex® for one, two and four weeks in pruritus gravidarum (PG) (n=8) and hepatosis of pregnancy (HP) (n=12) Mean±S E M*

N S =not significant

| Duration of treatment | *n* | Pruritus gravidarum: Bilirubin (mg/100 ml) | Alkaline phosphatase (Buch Units) | SGOT (Units/l) | SGPT (Units/l) | Simplastin A (%) | *n* | Hepatosis of pregnancy: Bilirubin (mg/100 ml) | Alkaline phosphatase (Buch Units) | SGOT (Units/l) | SGPT (Units/l) | Simplastin A (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I Pre treatment values | 8 | 0.4±0.1 | 7.8±1.3 | 15±5 | 12±5 | 129±15 | 12 | 1.2±0.2 | 16±0.7 | 64± 9 | 87±16 | 158±11 |
| II One week | 6 | 0.5±0.1 | 9.2±1.4 | 19±5 | 16±7 | 112±10 | 8 | 1.6±0.6 | 18±1.6 | 89± 9 | 92±13 | 158±17 |
| III Two weeks | 6 | 0.4±0.1 | 7.0±1.1 | 19±7 | 16±6 | 112± 8 | 9 | 1.8±0.5 | 17±1.8 | 71±13 | 93±23 | 143±15 |
| IV Four weeks | 5 | 0.5±0.1 | 7.0±1.0 | 12±2 | 10±2 | 137±17 | 10 | 1.7±0.4 | 22±2.5 | 61±11 | 91±19 | 129±11 |
| I vs II | P | N S | N S | N S | N S | N S | | N S | N S | N S | N S | N S |
| I vs III | P | N S | N S | N S | N S | N S | | N S | N S | N S | N S | N S |
| I vs IV | P | N S | N S | N S | N S | N S | | N S | <0.05 | N S | N S | <0.05 |

Table V *Effect on serum lipids and lipoproteins during oral treatment with Secholex® for 1, 2 and 4 weeks in pruritus gravidarum (PG)(n=8) and hepatosis of pregnancy (HP) (n=12) Mean±S E M*

N S =not significant CH=cholesterol PL=phospholipids TG=triglycerides LP=lipoproteins

| Duration of treatment | *n* | Pruritus gravidarum: CH | PL (mg/100 ml) | TG (mg/100 ml) | α LP CH | LP X (arb units) | *n* | Hepatosis of pregnancy: CH | PL (mg/100 ml) | TG (mg/100 ml) | α LP CH | LP X (arb units) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I Pre treatment values | 8 | 256±15 | 297±18 | 205±24 | 74− 8 | 2.4±0.2 | 12 | 316±23 | 338±26 | 271±14 | 59±5 | 3.3±0.2 |
| II One week | 6 | 265±16 | 307±18 | 197±23 | 87±11 | 2.2±0.3 | 8 | 286±12 | 316±12 | 262±12 | 56±4 | 3.1±0.3 |
| III Two weeks | 6 | 239± 8 | 279±10 | 173±24 | 77± 4 | 1.8±0.3 | 9 | 290±12 | 318±15 | 290±30 | 56±4 | 3.6±0.4 |
| IV Four weeks | 5 | 239± 4 | 277± 3 | 162±14 | 80± 3 | 1.0±0.4 | 10 | 293±23 | 331±34 | 285±32 | 56±5 | 2.4±0.4 |
| I vs II | P | N S | N S | N S | N S | N S | | <0.05 | N S | N S | N S | N S |
| I vs III | P | N S | N S | N S | N S | N S | | N S | N S | N S | N S | N S |
| I vs III | P | N S | N S | N S | N S | N S | | N S | N S | N S | N S | <0.05 |

Table VI. *Serum iron, total iron binding capacity (TIBC) and folic acid in whole blood and serum in cholestasis of pregnancy (n=6) before and after oral treatment with Secholex® (5 g t.i.d.) for 4 weeks. Mean ± S.E.M.*

N.S. = not significant

| Duration of treatment | Serum iron (μg/100 ml) | TIBC (μg/100 ml) | Folic acid whole blood (ng/ml) | Folic acid serum (ng/ml) |
|---|---|---|---|---|
| I Pretreatment values | 107±24 | 619±69 | 179±12 | 5.7±0.8 |
| II Four weeks | 119±24 | 570±76 | 132±10 | 3.9±0.3 |
| I vs II *P* | N.S. | N.S. | N.S. | <0.05 |

most marked (100%) in the group of women with PG.

*Obstetric data* (Table I). Normal deliveries occurred in the 35th–43rd gestational week (mean 39.4) in 16 (80%) of the women. One vacuum extraction and three Caesarean sections were performed on obstetric indications. One twin and two breech deliveries were encountered.

*Pediatric aspects* (Table I). Placental and birth weights were within normal limits, mean 583 g and 3404 g, respectively. Immaturity was diagnosed in two babies delivered in the 34th and 35th gestational week, respectively. There was no perinatal mortality.

*Liver function tests* (Table IV). In women with PG no changes in liver function tests were observed during the administration of Secholex.

In women with HP serum alkaline phosphatase increased by 35% ($p<0.05$) after 4 weeks, whilst Simplastin A values concomitantly fell ($p<0.05$).

*Serum lipids and lipoproteins* (Table V). In HP serum cholesterol decreased by 10% ($p<0.05$) after one week of Secholex administration, but thereafter tended to rise again. LP X levels were lower ($p<0.05$) in the women with HP treated with Secholex for 4 weeks.

*Hematological data* (Table VI). The serum concentration of folic acid decreased ($p<0.05$) after four weeks of treatment in 6 patients. No other changes were recorded in the hematological data: serum iron, TIBC or whole blood folic acid.

*Fatty acid composition in serum phosphoglycerides (GPL) and lecithin (PC).* The fatty acid pattern of serum lecithin in CP is characterized by a low 16:0 (palmitic acid) and a high 18:1 (oleic acid) content when compared with the pregnant control series (13).

The oral administration of Secholex for 2 weeks resulted in a higher ($p<0.05$) 16:0 (palmitic acid) and a lower ($p<0.001$) 18:0 (stearic acid) relative (and absolute) fatty acid content of serum lecithin. These changes in the fatty acid composition approached that of the control series. No effect on the essential fatty acids 18:2 (linoleic acid) and 20:4 (arachidonic acid) was noted during treatment with Secholex (Table VII).

## DISCUSSION

Encouraging results have earlier been presented on the relief of pruritus in cholestasis of pregnancy (CP) after oral administration of another anion exchange resin, cholestyramine (8, 11, 17). In the present study 31 women with CP verified by the presence of lipoproteins X (LP X) in their sera were given a new anion exchange resin, Secholex® for up to 8 weeks in an attempt to relief pruritus. In 20 women continuing the study for at least one week a good relief in pruritus was obtained in 38% and some relief in an addition 42%. The group of women with CP was divided according to severity of their disease into one group *with pruritus gravidarum (PG)* and another group with hepatosis of pregnancy (HP), *the differentiation* being based on liver function tests (cf. Materials and Methods). The best effect was achieved in the milder group, PG, where *all patients (n=8)* experienced relief from pruritus. Although a placebo effect is always experienced by the administration of a drug, this effect is never as high as 80% and would not affect laboratory variables. A double blind study with Secholex and a placebo was not possible as an insufficient number of cases with CP was available for study.

Table VII *Composition of major fatty acids in serum lec[illegible] cholestasis of pregnancy (n=11) (weeks of gestation mean 34 3 range 24–40) before and after two and four weeks of oral treatment with Secholex® (5 g t i d ) compared with control series (normal pregnancies n=20 weeks of testation mean 33 6 range 33–37)*

Figures are given in mole per cent of methyl esters

| | | 16 0 | 16 1 n 7 | 18 0 | 18 1 n 9 | 18 2 n 6 | 20 3 n 6 | 20 4 n 6 | 22 6 n 3 | 18–22 n 6 | 18 2/20 4 | Fatty acids (mg/100 ml) | Lecithin (mg/100 ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I Pre treatment values | X | 33 7 | 1 3 | 10 5 | 14 2 | 24 9 | 3 2 | 6 1 | 4 2 | 34 4 | 4 2 | 102 | 244 |
| n=11 | S E M | 0 56 | 0 09 | 0 43 | 0 70 | 0 80 | 0 14 | 0 35 | 0 29 | 0 80 | 0 33 | 7 39 | 12 3 |
| II Two weeks | X | 34 9 | 1 2 | 9 6 | 14 5 | 25 2 | 3 2 | 6 0 | 3 7 | 34 7 | 4 4 | 101 | 242 |
| n=11 | S E M | 0 39 | 0 10 | 0 37 | 0 80 | 0 76 | 0 23 | 0 31 | 0 37 | 0 84 | 0 33 | 6 92 | 11 5 |
| III Four weeks | X | 33 9 | 1 3 | 9 9 | 14 6 | 25 2 | 3 3 | 6 0 | 4 0 | 34 8 | 4 4 | 117 | 270 |
| n=6 | S E M | 1 07 | 0 13 | 0 63 | 1 11 | 0 93 | 0 13 | 0 49 | 0 42 | 1 35 | 0 26 | 7 04 | 11 7 |
| IV Normal pregnancy | X | 37 4 | 1 0 | 9 5 | 17 7 | 24 9 | 2 8 | 5 7 | 4 0 | 33 7 | 4 6 | 111 | 261 |
| n=20 | S E M | 0 42 | 0 04 | 0 72 | 0 25 | 0 44 | 0 14 | 0 25 | 0 24 | 0 48 | 0 28 | 4 17 | 9 81 |
| I vs II | P | <0 05 | N S | <0 001 | N S | N S | N S | N S | N S | N S | – | N S | N S |
| I vs III | P | N S | N S | N S | N S | N S | N S | N S | N S | N S | – | N S | N S |
| I vs IV | P | <0 001 | <0 01 | <0 05 | N S | N S | N S | N S | N S | N S | – | N S | N S |
| II vs IV | P | <0 01 | N S | N S | <0 05 | N S | N S | N S | N S | N S | – | N S | N S |

The high rate of withdrawal was primarily due to gastro-intestinal difficulties ($n$=6) particularly constipation On the other hand no serious side effects were met with during the total period of Secholex administration

Anion exchange resins form a non absorbable complex with bile acids in the intestine and thereby facilitate the excretion of bile acids in faeces The best effect on pruritus by Secholex was seen as might be expected in the milder form of cholestasis of pregnancy where bile acid secretion into the intestine is only partially blocked In HP with a more extensive blocking of bile secretion into the intestine—and more advanced liver damage—less improvement in the pruritus was experienced

In earlier studies on cholestatic patients (5) variations in serum bile acid concentrations (corresponding to successively administering and discontinuing choles yramine) were observed to correlate with changes in the intensity of pruritus The possibility that the degree of pruritus is related to serum bile acid concentration is further supported by the present data in PG and HP patients respectively The influence of anion exchange resins on other non identified compounds such as steroids (20) as an explanation for the relief of pruritus cannot be ruled out

The bile acid sequestering action of the anion exchange resin would be expected to interfere with the absorption of fat and fat soluble vitamins *In the present study* vitamins were administered parenterally to prevent deficiency Although no vitamins K was administered bleeding anomalies were not experienced After 4 weeks of Secholex administration no change in whole blood folic acid was seen A decrease in serum folic acid also seen after the administration of cholestyramine (27) suggests a possible interference with the absorption of folic acid in the proximal *portion of jejunum* The exact interaction of Secholex with folic acid is not revealed by the present study It should be remembered however that during treatment with biguanides (?) serum folic acid levels also appeared to be an early and sensitive measure of folic acid depletion

No steatorrhoea and little diarrhoea was encountered during the treatment periods The stability of the relative and absolute amounts of essential linoleic (18 2) and arachidonic (20 4) acids in serum lecithin during Secholex intake

indicates little interaction with intestinal fat absorption These results however do not rule out the possibility that Secholex intake exceeding 5 g t i d for more than 4 weeks might impair intestinal fatty acid absorption

The effect of Secholex on the fatty acid composition of serum lecithin suggests that it influences the basic defect of the disease Secholex increased the characteristically low content of palmitic acid (16 0) and reduced the content of stearic acid (18 0) The serum cholesterol increase during normal pregnancy most marked in the first and second trimester is assumed to be of physiological importance In CP the serum cholesterol increase is even more marked (15) primarily due to the appearance in serum of an abnormal lipoprotein LP X rich in free cholesterol In the present study the administration of Secholex resulted initially in a slight reduction in serum cholesterol but as a whole little effect on serum lipids was ecountered

During Secholex administration none of the liver function tests were lowered On the contrary alkaline phosphatase values were further increased in the HP group This is in line with the findings of Gustafson & Lanner (9) who during administration of Secholex to hypercholesterolemic subjects noted a transient increase in alkaline phosphatase values

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*Submitted for publication March 27 1974*

Acta Obstet Gynecol Scand 54 341–346 1975

# CORRELATION BETWEEN HUMAN CHORIONIC SOMATOMAMMOTROPIN AND PLACENTAL WEIGHT

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*Abstract* Human chorionic somatomammotropin concentration in serum (S-HCS) during the latter half of pregnancy was measured by radioimmunoassay and correlated to the placental weight in two group of normal healthy pregnant women In one group 7–8 samples from 47 women were examined which gives a longitudinal series In the other group single samples from each of 346 pregnant women were examined which gives a cross sectional series Both groups were randomized on the basis of a prospective selection The mean values of S HCS in each week of gestation were almost identical in the two groups showing a steady increase until 37–38 weeks and a subsequent decrease In the longitudinal series there was a positive correlation between the S-HCS and placental weight after 37 weeks gestation but not before that time Before 37 weeks gestation the ratio S-HCS/placental weight was significantly higher with small placentae than with large placentae This difference between small and large placentae disappeared after 37 weeks These results point to the existence of some regulatory mechanism tending to keep the S HCS concentration within certain limits independent of placental weight This mechanism appears to be lost after 37 weeks of gestation when the S-HCS concentration starts to correlate with placental weight

The discovery that Human Chorionic Somatomammotropin (HCS) is produced solely in the syncytiotrophoblast layer of the placenta (12) has led to the assumption that the serum HCS concentration might be an index of placental function It has been shown in many ways that there is a connection between S HCS and placental function but it seems difficult to differentiate between the patient with placental insufficiency and the patient with a poor obstetric outcome for other reasons (2) If we want to use the term placental function test we must clarify whether the S-HCS concentration gives us information about the metabolic function or the growth or the mass of the placenta This is necessary in order to evaluate the significance of either a series of decreasing S HCS values or of a single low value (18 22) Until now no justification has been evident for allowing such findings to have therapeutic consequences

Many authors believe that the secretion is probably controlled by either a hormone or a metabolic product or intracellularly from the placenta itself (3) If the secretion is entirely extracellular controlled and there are wide fluctuations in secretion rate the variation of the results (4 6 10 13) when related to the placental weight seems of little importance

The present investigation will attempt further clarification of the correlation between the serum concentration of HCS and placental weight

## MATERIAL

The material consists of two groups of pregnant women selected prospectively using the following criteria they were born in Denmark and resident in the municipality of Odense aged between 17 ½ and 35 Menstrual periods were regular with less than 6 days variation and length of the cycle between 21 and 35 days The date of the last menstrual period was known and reliable with at least 4 normal periods after cessation of oral contraception Women with an obstetric history of one serious or two less serious complications were excluded The present pregnancy was normal at the antenatal examinations Smoking amounted to less than 10 cigarettes a day

One group consists of 47 pregnant women selected at random From these a blood sample was drawn at each visit to the atenatal clinic 23 were examined from 22 weeks of gestation onwards (all weeks are completed) 24 women from 37 weeks onwards 38 women had a sample drawn

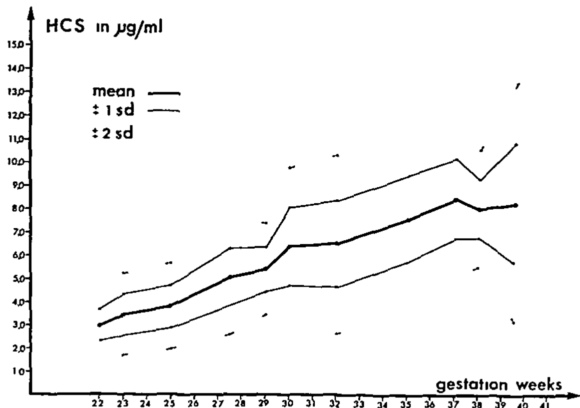



*Fig 1* Mean values of S HCS with ±1 S D and ±2 S D from week 22 to week 40 The graph includes 264 women lected before and after delivery

after the rupture of the membranes but before commencement of the second stage contractions

The other group is a cross sectional series originally comprising 346 pregnant women One blood sample was drawn from each of them at some time between 22 weeks of gestation and the delivery No sample was drawn after rupture of the membranes in this series After delivery women who gained less than 7 kg during the pregnancy or who gave birth to a seriously malformed baby were withdrawn from the study Also all cases in which the weight of either infant or placenta was outside 2 S D of a reference group of the Odense Obstetrical Department were included (i e 2620–4390 g and 283–609 g respectively) In all 82 mothers were excluded leaving a total of 264 women

## METHODS

Before the weighing of the placenta the umbilical cord and membranes were cut off and the placenta gently squeezed and rinsed with saline Antecubital venous blood samples were drawn and serum was stored at minus 70°C until S-HCS could be determined in duplicate with HPL Immunoassay Kit" from the Radiochemical Centre Amersham England (8 24)

The day to-day reproducibility of HCS in sera containing from 5 6 to 7 7 mg/litre showed a coefficient of variation from 5 1 to 7 5%

## RESULTS

In Fig 1 the S HCS values from the cross section group are depicted with mean values and standard deviations in relation to gestational age The mean values gradual increase until 2–3 weeks before delivery and after that time they decrease The peak value for the cross section material is 8 45 mg/l (1 S D =1 71) at 38 weeks gestation In the longitudinal series (not depicted) this value is 8 38 mg/l (1 S D =1 50) and is found at 37 weeks gestation The decrease from 37–41 weeks was evaluated by the Sign test (15) and is significant ($p<0 05$)

Of the 47 placentae in the longitudinal series the weights of seven were above +1 S D and eight were below −1 S D The S HCS of these two groups are shown in Fig 2*a* The rank sum test (16) confirms that the values of HCS relating to the large placentae do not differ from those relating to the small placentae before 38 weeks gestation After that time women with large placentae have higher S HCS than those with small placentae In Table I left side are shown the results of a week to-week

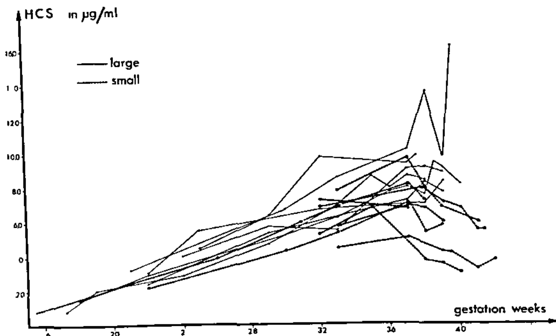



*Fig 2a* S HCS values from week 15 to week 42 from the longitudinal series The lines represent the values from women with the 7 largest and the 8 smallest placentae respectively

correlation analysis for all 228 S HCS measurements in relation to the 47 placental weights at birth The results are the same as those seen in Fig 2*a* The correlation coefficient increases from 38 weeks until delivery ($r$=0 38–0 62) The samples marked X in Table I are drawn during the first stage of labour They show correlation patterns similar to the late gestation samples

The ratios S HCS/placental weight of the 7 large and the 8 small placentae in relation to gestational age are shown in Fig 2*b* It is seen that this ratio is higher for the small placentae until 38–39 weeks of gestation After that time there is no difference between small and large placentae This was confirmed by a correlation analysis of the two variables the ratio S HCS/placental weight and the 47 placental

Table I *Statistical evaluation of S HCS and the ratio S HCS/placental weight and their correlation to placental weights from 47 women*

| | | Correlation between HCS in mg/l and placental weight at birth | | | Correlation between placental weight at birth and the ratio HCS in mg/l divided by placental weight in gram (×10⁻⁹) | | |
|---|---|---|---|---|---|---|---|
| Gestation week | Number of samples | HCS mean | Correlation coefficient | Significance | Mean | Correlation coefficient | Significance |
| 29–31 | 25 | 5 6 | −0 076 | NS | 118 | −0 617 | $p<0$ 001 |
| 32 | 16 | 7 2 | 0 390 | NS | 16[illegible] | −0 735 | $p<0$ 01 |
| 33 | 19 | 6 7 | 0 154 | NS | 16[illegible] | −0 702 | $p<0$ 001 |
| 34–36 | 20 | 7 9 | −0 055 | NS | 173 | −0 765 | $p<0$ 001 |
| 37 | 33 | 8 4 | 0 118 | NS | 190 | −0 691 | $p<0$ 001 |
| 38 | 31 | 7 8 | 0 379 | $p<0$ 05 | 177 | −0 527 | $p<0$ 01 |
| 39 | 27 | 7 4 | 0 541 | $p<0$ 01 | 171 | −0 418 | $p<0$ 05 |
| 40–41 | 19 | 6 9 | 0 623 | $p<0$ 01 | 154 | −0 431 | NS |
| X | 38 | 7 2 | 0 401 | $p<0$ 05 | 163 | −0 244 | NS |

X represents samples taken during delivery

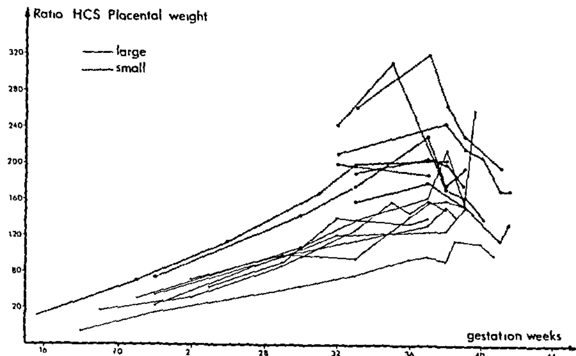



*Fig 2b* The ratio S-HCS/placental weight in the same period and for the same placentae as in Fig 2*a*

weights There is a significant negative correlation until 38–39 weeks as shown in Table I right side

The cross sectional material consisting of 346 samples taken from 346 women was similarly analysed In this series a positive correlation between S HCS and placental weight was found from 30 weeks of gestation with an increasing coefficient toward the time of delivery The ratio S HCS/placental weight in relation to placental weight showed good agreement with the longitudinal series By 22 weeks of gestation there was already a significant negative correlation with $r = -0.66$ ($p < 0.001$)

In order to eliminate the bias of varying lengths of time from a certain gestational week until delivery a correlation analysis was made using gestational age in weeks before delivery instead of dating from the last menstrual period The correlation between S HCS and placental weight showed in this analysis the same patterns and the same magnitudes as shown in the left side of Table I

In the longitudinal series the S HCS was compared with the birth weights of the infants Numbers and intervals were the same as those shown in Table I No correlation was found until 36 weeks A positive correlation was found after that time including the samples drawn during the first stage of labour The $r$ values are a little lower than those correlating to placental weight

## DISCUSSION

The position course and numerical peak value of the curve in Fig I are in agreement with Genazzani s (4) recently published results

The increasing correlation between S HCS and placental weight through gestation can possibly explain why some investigators (13 17) find low or no correlation early in pregnancy while others (11 14) find a very high correlation at term However the significance of these last mentioned results is difficult to evaluate because of the composition of the various series

In Fig 2*b* the negative correlation between placental weight and the ratio S HCS/placental weight is seen until 37 weeks Provided the elimination rate is unchanged during the gestation period the results will indicate that small placentae produce more HCS per weight unit placental tissue than do large placentae Thus the scatter of individual S-HCS values is limited up to 37 weeks This suggests that there exists a regulatory mechanism for the S HCS controlling either production or elimination or both

If the interpretation of our results is correct there is a strong indication that the secretion of HCS is regulated and that this regulation ceases to operate about 2 weeks before delivery Josimovich s (5) assumption that the mass of the placenta alone de termines the production of HCS hardly seems justified on that basis Whether the supposed feed back mechanism involves a hormone a metabolic product or other factors we cannot venture to guess at the present time

Some investigators (1 19 23) have demonstrated that glucose infusion results in a slight increase in S HCS Insulin administration and prolonged star vation have the opposite effect Spellacy (20) re cently pointed out that S HCS levels and insulin requirements do not correlate in pregnant diabetics He concludes that carbohydrate metabolism is of minor importance for the regulation of HCS secre tion The variations in S HCS and the ratio S HCS/placental weight are so large that alterations in carbohydrate metabolism cannot explain them

We have found that the correlation between HCS and foetal weight is not significant until 37 weeks of gestation and this is in agreement with the findings of Genazzani (4) The fact that the correlation coeffi cient to foetal weight is smaller than that to placental weight suggests that the production of HCS is more closely related to placental tissue than to foetus

Our conclusions are open to some criticisms Firstly one might question the assumption that S HCS is a correct measure of the production of HCS It probably is even if alterations in the elimi nation rate blood volume and distribution in the tissues may affect the serum level to a certain de gree One could also criticize the differences of correlation patterns in the two series However as pointed out so clearly by Tanner (21) the two series differ one being cross sectional and the other lon gitudinal and they cannot be expected to show identical results

A third criticism is the justification for correlating S HCS measured many weeks before delivery to the placental weight at birth The correlation between the ratio HCS/placental weight and placental weight is most marked in the early weeks of pregnancy (?2–30 weeks) which would hardly be the case if it was meaningless to correlate the two values meas ured over a span of time Furthermore if all placentae grow in a similar way during the last 10 weeks of pregnancy the correlations would not change whether or not a correction is made using a placental growth curve If we imagine that the placentae stop growing at widely different times before delivery the correlation analysis will be dis turbed in an incalculable way According to Kloosterman (7) this is unlikely and it has been shown in animal experiments (9) that the placenta has a rather constant life cycle and stops growing some time before delivery

In conclusion the results seem to show that the production of HCS during the last 2–3 weeks of pregnancy is without control and dependent only upon the mass of the placenta Prior to 37–38 weeks there seems to be an unknown controlling factor

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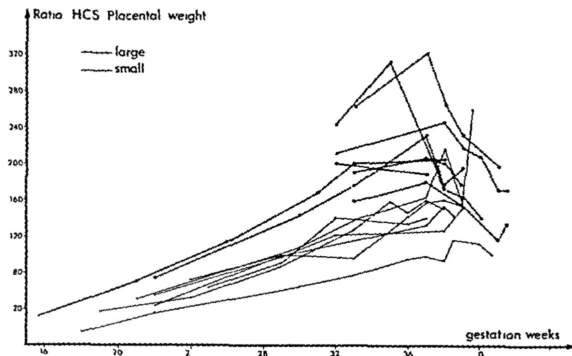



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Acta Obstet Gynecol Scand 54 347–356 1975

# PATTERNS OF FREE FATTY ACIDS GLYCEROL D β HYDROXYBUTYRATE AND INSULIN IN PREGNANT WOMEN AND THEIR NEWBORN INFANTS

*Effects of a Low and a High Insulin Response to Glucose in the Mothers*

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*Abstract* Twenty eight healthy women 17 with high and 11 with low insulin response to glucose but with normal glucose tolerance were followed throughout pregnancy Plasma FFA glycerol and D-β hydroxybutyrate as well as plasma insulin and glucose in blood were determined before and during a glucose infusion test (GIT) in each trimester and after pregnancy In 13 infants of high insulin responders (IHR) and 10 infants of low responders (ILR) an intravenous glucose tolerance test (IVGTT) was performed and the above lipid parameters were studied at birth and during the IVGTT The low responder group was postulated to consist mainly of prediabetic individuals (8) Their infants have previously been shown to have an increased glucose assimilation rate at IVGTT (12 13) as has been shown for infants of diabetic mothers

There was little difference between the two groups of mothers except for the insulin levels during the GIT in non pregnant and early pregnant subjects which were considerably lower in the low responders They all had decreased fasting levels of FFA glycerol and D-β hydroxybutyrate in mid pregnancy and normal values in late pregnancy The ILR showed the same changes in FFA and glycerol as the IHR but their D-β hydroxybutyrate levels were higher at birth than those of the IHR and lower after birth Another difference found was the correlation between birth weight and fasting insulin (and to some extent the insulin level at birth) in the ILR group which was not found in the IHR Apart from those differences the ILR and the IHR seemed to handle their fat metabolism in a similar way in the early neonatal perinatal period.

It has been shown earlier by Cerasi & Luft (7) that prediabetic individuals (defined as healthy monozygotic twins of diabetic patients) have a very low insulin response to a continuous glucose infusion (GIT) This response is identical to that seen in diabetic subjects A low insulin response is found in 15–20% of healthy subjects (6) and it has been considered to be characteristic of—even if not necessarily pathognomonic of—the prediabetic individual

In a previous study a group of healthy women with such a low insulin response (LR) and a group with a high response (HR) were followed during pregnancy (11) The insulin response of the LR increased in late pregnancy in a similar way to that which has been reported by several authors for normal pregnancy (3 38 42) but it was consistently lower than that of the HR Fasting levels of free fatty acids (FFA) in plasma have often been reported to be elevated in normal late pregnancy (3 4 15 17 23 29) as compared with non pregnant subjects and the fasting levels of FFA glycerol and D-β hydroxybutyrate in pregnant diabetic women have been shown to be higher (23 37) It was therefore considered to be of interest to study these parameters in the above group of LR—or potential prediabetic individuals—and to see whether they differed in their lipid metabolism as well as in their glucose–insulin interrelationship

Full term infants have low plasma values of FFA glycerol and D-β-hydroxybutyrate at birth During the first day of life these parameters increase rapidly and markedly (9 33 39) Infants of diabetic mothers (IDM) on the other hand show a continued suppression of FFA levels during the first few hours while glycerol increases normally (28 36) The IDM are well known to

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Table II *Fasting levels of FFA glycerol D β-HBA insulin and glucose in high responders (HR) and in low responders (LR)*

Mean values are given for the non pregnant state (10-45 weeks after delivery) and for pregnancy trimesters I-III Significance of changes within the group is indicated with (p<0 05) (p<0 01) or (p<0 001)

| Parameter | | Non pregnant | Pregnancy trimester I | II | III |
|---|---|---|---|---|---|
| FFA mM/l | | | | | |
| HR | x | 0 42 | 0 40 | 0 37 | 0 41 |
| | S E M | 0 04 | 0 04 | 0 03 | 0 04 |
| | n | 13 | 15 | 17 | 17 |
| LR | x | 0.52 | 0 37 | 0 32 | 0 37 |
| | S E M | 0 06 | 0 03 | 0 0? | 0 04 |
| | n | 11 | 11 | 13 | 10 |
| Glycerol mM/l | | | | | |
| HR | x | 0 115 | 0 077 | 0 066 | 0 081 |
| | S E M | 0 019 | 0 008 | 0 006 | 0 006 |
| | n | 13 | 15 | 17 | 17 |
| LR | x | 0 112 | 0 076 | 0 067 | 0 084 |
| | S E M | 0 016 | 0 009 | 0 006 | 0 014 |
| | n | 11 | 11 | 13 | 10 |
| D-β HBA mM/l | | | | | |
| HR | x | 0 062 | 0 032 | 0 041 | 0 061 |
| | S E M | 0 017 | 0 004 | 0 006 | 0 009 |
| | n | 13 | 15 | 17 | 17 |
| LR | x | 0 079 | 0 068 | 0 040 | 0 065 |
| | S E M | 0 013 | 0 0?1 | 0 005 | 0 012 |
| | n | 11 | 11 | 13 | 10 |
| *Insulin* μU/ml | | | | | |
| HR | x | 16 7 | 17 8 | 19 4 | 22 1 |
| | S E M | 1 0 | 1 3 | 1 8 | 1 5 |
| | n | 18 | 15 | 18 | 18 |
| LR | x | 14 9 | 16 3 | 17 2 | 18 6 |
| | S E M | 0 8 | ? 1 | 1 2 | 1 7 |
| | n | 13 | 11 | 13 | 12 |
| Glucose mg/100 ml | | | | | |
| HR | x | 72 9 | 7? 6 | 67 4 | 65 9 |
| | S E M | 1 6 | 2 0 | 1 8 | 2 7 |
| | n | 18 | 15 | 18 | 18 |
| LR | x̄ | 77 5 | 71 6 | 62 7 | 65 8 |
| | S E M | 2 8 | 2 1 | 2 0 | 2 3 |
| | n | 13 | 12 | 13 | 12 |

## MATERIAL AND METHODS

Twenty-eight healthy women were repeatedly subjected to a GIT during and after pregnancy During these test blood samples were drawn for analyses of blood glucose and plasma concentrations of FFA glycerol D-β-hydroxybutyrate and insulin Their insulin response to glucose during the GIT was computed according to the technique described by Cerasi & Luft (6) The changes in insulin response during pregnancy have been discussed in detail in an earlier paper (11) but they will be referred to briefly below

The women were defined as high or low insulin responders (HR and LR) according to their immediate insulin response in the non pregnant state that is their insulin release in relation to blood glucose during the first 10 min Fasting levels of FFA glycerol and D-β hydroxybutyrate were determined in 17 HR and 11 LR during their 18 and 13 pregnancies Changes in the same parameters during the GIT were studied in all LR and in 12 HR

In 25 out of the 31 infants the levels of glucose insulin FFA glycerol D-β hydroxybutyrate and growth hormone (HGH) were studied at birth and during the IVGTT that was performed at 4-24 hours of age Before the IVGTT informed consent was obtained from the parents

### *The glucose infusion test (GIT)*

The mothers were tested whenever possible in the 9th 2?nd and 36th weeks of pregnancy and also at 7-44 weeks post partum After an overnight fast 500 mg of glucose per kg body weight were injected intravenously and thereafter a constant dose of 20 mg per kg and minute was infused for one hour The plasma concentration of glucose was kept at approximately 200-300 mg/100 ml blood Venous blood samples were collected at 0 5 10 ?0 30 40 50 60 80 100 and 120 min after the start of the infusion The samples were immediately centrifuged and plasma was separated and frozen for later analysis

### *The glucose tolerance test (IVGTT)*

In the infants an IVGTT was performed at 4-?4 hours of age the mean age being 13 4±2 0 hours (mean and S E M ) for the 10 ILR and 16 4±1 4 hours for the 13 IHR The infants were not fed prior to the test and none of the mothers received any glucose infusion during the delivery The infants were kept on a heated mattress and covered with a towel 1 5 g of glucose per kg body weight (30 or 50% solution) was slowly injected via either a peripheral vein or the umbilical artery the mean time required for the injection was 7 8 min (range 3-14 min) Blood samples of ?-3 ml were drawn from a catheter in the umbilical vein at 0 5 10 20 and 60 min from the start of the injection The tip of the catheter was passed up approximately 10-12 cm from the umbilicus Capillary blood samples of 0 1 ml for glucose determinations were drawn at 0 min and then every 10 min for one hour Glucose in the umbilical cord at birth was determined in plasma all other glucose analyses were performed on whole blood

### *Laboratory methods*

Plasma FFA were determined using the colorimetric micromethod of Laurell & Tibbling (25) glycerol by the enzymatic fluorometric micromethod of the same authors (24) and D-β hydroxybutyrate by the fluorometric micromethod of Persson (34) Plasma insulin was determined using the radioimmunoassay of Hales & Randle (21) and HGH by a similar radioimmuno-

Table I *Clinical data on 28 women (17 high insulin responders and 11 low responders) and their infants*
NP=normal vaginal delivery VE=termination by vacuum extraction and CS=caesarean section M=male and F=female infant

| | Maternal | | | | | | Infant s | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Subj No | Age at del (y ) | Parity | Weight before pregn (kg) | Height in cm | Durat of labour (h) | Mode of delivery | Gestational age (w ) | Age at IVGTT (h) | Sex | Body weight (g) |
| *High insulin responders* | | | | | | | *IHR* | | | |
| 1 | 31 | 1 | 43 | 158 | 22 | NP | 38 | 12 | M | 2 560 |
| 2 | 25 | 0 | 54 | 163 | 9 | NP | 41 | – | M | 3 230 |
| 3 | 27 | 0 | 64 | 168 | 7 | VE | 40 | 15 | F | 3 240 |
| 4 | 34 | 1 | 79 | 172 | 11 | VE | 40 | 22 | F | 4 230 |
| 5 | 34 | 2 | 65 | 175 | 4 | NP | 41 | 22 | F | 4 470 |
| 6 | 27 | 0 | 68 | 166 | 6 | NP | 40 | – | M | 3 860 |
| 7 | 30 | 2 | 53 | 167 | 6 | NP | 40 | – | F | 4 150 |
| 8 | 26 | 1 | 61 | 174 | 5 | NP | 41 | 16 | M | 3 380 |
| 9 | 27 | 0 | 60 | 172 | 5 | NP | 40 | – | F | 3 370 |
| 10 | 27 | 0 | 54 | 163 | 6 | VE | 41 | 20 | M | 4 470 |
| 11 | 27 | 0 | 65 | 171 | 7 | NP | 41 | – | F | 3 450 |
| 12 | 26 | 0 | 65 | 175 | 11 | NP | 41 | 16 | F | 4 410 |
| 13 | 34 | 0 | 64 | 170 | 26 | NP | 41 | 10 | F | 3 600 |
| 14 | 34 | 0 | 47 | 158 | 7 | NP | 41 | 23 | F | 3 030 |
| 16 | 29 | 0 | 54 | 162 | 8 | NP | 40 | 22 | F | 3 570 |
| 17 a | 25 | 0 | 55 } | | 23 | VE | 40 | 10 | M | 3 950 |
| 17 b | 27 | 1 | 58 } | 172 | 8 | NP | 41 | 15 | M | 4 210 |
| 18 | 25 | 0 | 65 | 165 | 4 | NP | 41 | 15 | M | 4 710 |
| $\bar{x}$ | 28 6 | | 59 7 | 167 7 | 9 7 | | 40 4 | 16 4 | | 3 708 |
| S E M | 0 8 | | 2 0 | 1 3 | 1 6 | | 0 2 | 1 4 | | 155 |
| *n* | 18 | | 18 | 17 | 18 | | 18 | 13 | | 18 |
| *Low insulin responders* | | | | | | | *ILR* | | | |
| 21 | 31 | 1 | 61 | 162 | 2 | NP | 41 | – | M | 3 600 |
| 22 | 25 | 0 | 45 | 155 | 9 | NP | 40 | 4 | F | 3 090 |
| 23 | 24 | 1 | 49 | 165 | 6 | NP | 38 | 21 | M | 3 370 |
| 24 a | 22 | 0 | 47 } | | 10 | CS | 41 | – | M | 3 740 |
| 24 b | 24 | 1 | 48 } | 159 | 0 | CS | 39 | 6 | M | 3 410 |
| 25 | 31 | 0 | 57 | 171 | 12 | NP | 40 | 11 | F | 3 110 |
| 26 | 25 | 0 | 55 | 168 | 7 | NP | 43 | 19 | M | 3 770 |
| 27 a | 27 | 0 | 50 } | | 8 | NP | 41 | – | M | 3 450 |
| 27 b | 28 | 1 | 50 } | 165 | 7 | NP | 40 | 23 | F | 3 480 |
| 28 | 31 | 0 | 48 | 160 | 5 | NP | 40 | 10 | M | 3 530 |
| 29 | 26 | 0 | 60 | 165 | 22 | VE | 41 | 15 | F | 3 900 |
| 30 | 29 | 0 | 60 | 172 | 16 | VE | 40 | 11 | F | 3 740 |
| 31 | 26 | 0 | 56 | 172 | 17 | NP | 42 | 14 | M | 3 700 |
| $\bar{x}$ | 26 8 | | 52 8 | 164 9 | 9 3 | | 40 5 | 13 4 | | 3 480 |
| S E M | 0 8 | | 1 5 | 1 7 | 1 7 | | 0 4 | 2 0 | | 70 |
| *n* | 13 | | 13 | 11 | 13 | | 13 | 10 | | 13 |

have hyperinsulinism and a higher elimination rate of glucose from blood after an intravenous glucose load (IVGTT) than the normal newborn infant (1 27) In the newborn infants of the low insulin responders (ILR) we found an increased glucose elimination rate at IVGTT (12 13) compared with that in the infants of the high responders (IHR) The ILR also to some extent showed signs similar to those of IDM like hyperexcitability hypoglycaemia and a pronounced postnatal weight loss (13) An investigation of the FFA glycerol and D-$\beta$ hydroxybutyrate levels at birth and during the IVGTT was therefore carried out to see whether they differed from the IHR also concerning their lipolysis lipid mobilization and oxidation as reflected in changes of these parameters

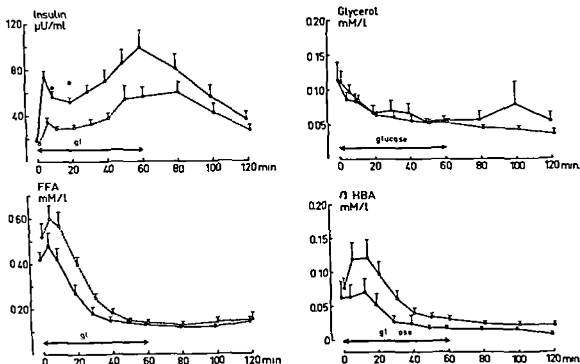



*Fig 1* Mean values of plasma insulin FFA glycerol and D-β HBA in 9 non pregnant high responders (whole lines filled circles) and 11 non pregnant low responders (broken lines open circles) during GIT Glucose was infused from 0 to 60 min Vertical bars denote S E M Stars denote significance of difference between the groups =$p<0.05$ =$p<0.01$ and =$p<0.001$

the increase in insulin and the decrease in FFA glycerol and D-β hydroxybutyrate during the test when performed in the non pregnant state There was a significant difference in insulin increase the high responders showing a more pronounced response both initially (the peak at 5 min) and during the secondary increase The changes in FFA glycerol and D-β hydroxy butyrate on the other hand are more or less identical in the two groups The maximum decrease in these parameters appears after one hour

*Changes during pregnancy in response to GIT* In Fig 2 the mean increment or decrement from the fasting plasma level is given for insulin FFA glycerol and D-β hydroxybutyrate Mean values for HR and for LR are given at 20 40 and 60 min after the start of the infusion for each test occasion The insulin response (here only the late response after 20 min and later is seen) increases markedly during pregnancy and in the third trimester the levels for the low responders are almost the same as those for the high responders FFA and glycerol decrease in a similar way in the two groups throughout pregnancy in spite of the differences in insulin levels found in early and late pregnancy The only significant difference between the groups concerning these parameters is the pronounced peak in FFA levels at 20 min seen in late pregnant high responders ($p<0.001$) The decrease in D-β hydroxybutyrate levels in the non pregnant and early pregnant LR is more pronounced than that seen in HR at the same time ($p<0.05$ at 40 min and $p<0.025$ at 60 min)

### *Infants*

*Clinical data* on the infants are given in Table I Two infants (Nos 24 *a* and 27 *a*) had an Apgar score of 6 at 2 min but at 10 min it had increased to 10 All other infants had an Apgar score of >8 The neonatal period was uneventful in all infants No significant differences were

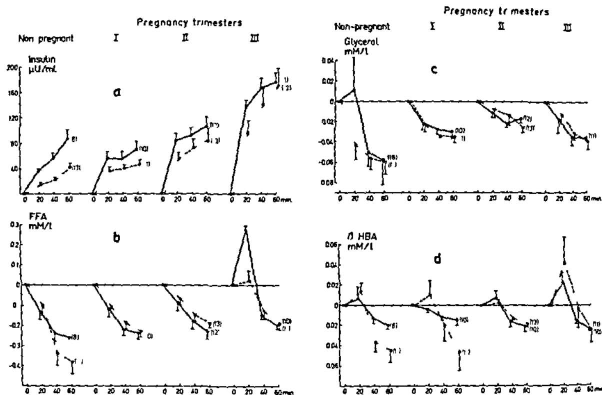



*Fig 2 a–d* Changes during pregnancy in the response of plasma insulin FFA glycerol and D-β HBA to GIT Mean values for increment or decrement from fasting value are given numbers within parenthesis denote number of individuals tested in the group during the pregnancy trimester in question For explanations of the symbols see legend to Fig 1

found in duration of labour gestational age birth weight or age at IVGTT

*At birth and before IVGTT mean values of insulin glucose FFA glycerol D-β-hydroxybutyrate and HGH* Mean values are given in Table III where the significance of differences between the groups is also indicated The increase of FFA and glycerol from birth to the IVGTT was significant ($p<0\ 001$) and identical for both groups D-β hydroxybutyrate also increased in IHR during the first day ($p<0\ 01$) while it *decreased* in the ILR ($p<0\ 025$) Glucose levels decreased markedly in both groups ($p<0\ 001$) but the increase seen in insulin and HGH levels was significant only for insulin in IHR ($p<0\ 05$)

The plasma levels of FFA glycerol and D-β hydroxybutyrate at birth were unrelated to the birth weight of the infant or to the duration of labour No significant correlation was found between the insulin level at birth and the birth weight even if the correlation within the ILR group was somewhat higher than in the IHR ($r=0\ 39$ $n=9$ compared to $r=0\ 18$ and $n=10$) The fasting level of insulin before the IVGTT and the birth weight were interrelated in the ILR group ($r=0\ 74$ $n=10$ $p<0\ 01$) but no corresponding relationship was seen in the IHR group or in the combined groups

A positive correlation was seen in the IHR group between the time elapsed between birth and IVGTT and the increase in D-β hydroxy butyrate levels ($r=0\ 77$ $n=11$ $p<0\ 01$) but not in the ILR group ($r=-0\ 14$ $n=9$ $p>0\ 05$)

*Changes in plasma levels during IVGTT* There was no difference between the groups in the mean values of insulin glucose FFA or glycerol (Fig 3) but the individual variation was great D-β hydroxybutyrate was significantly lower in ILR during the first 20 min

The increase in insulin levels at 20 min was significant in both groups ($p<0\ 01$ for ILR and $p<0\ 025$ for IHR using paired $t$ test) FFA glycerol and D-β hydroxybutyrate declined during the first hour after glucose injection except for

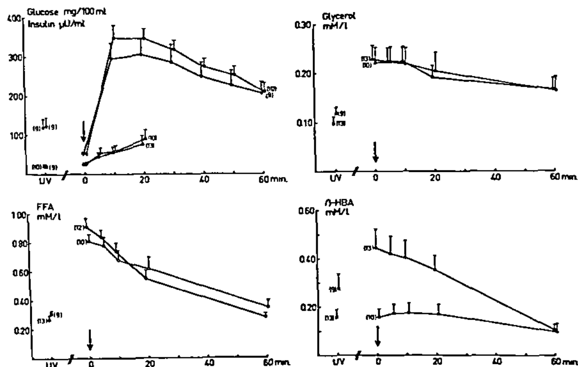



*Fig 3* Mean values for plasma insulin FFA glycerol D-β HBA (same symbols as in Fig 1 and 2) and blood glucose (IHR=filled triangles and ILR= open triangles) at delivery (UV) and at IVGTT Vertical arrow= start of glucose injection Stars denote significance of differences between groups (see Fig 1)

the last parameter in the ILR where the plasma levels were low already before the injection HGH increased moderately during the IVGTT The mean increase in 7 ILR was 22 9±7 6 and in 5 IHR 55 9±15 3 ng the difference was not significant neither was the increase within the groups

The total insulin increment at 20 min (expressed as microunits insulin per millilitre plasma per 20 min) was plotted against the corresponding decrements of FFA glycerol and D-β hydroxybutyrate but no correlation could be found

## DISCUSSION

The mothers in this prospective study did not show an increase in fasting levels of FFA glycerol and D-β hydroxybutyrate in late pregnancy compared to the levels in the non-pregnant state This is contrary to findings of other investigators who have reported elevated levels of FFA in healthy women during late pregnancy (3 4 15 17 23 29 40) However the findings here reported are in agreement with the findings of Persson & Lunell (37) Using the same methods for analysis as those used here they demonstrated unchanged levels of FFA glycerol and D-β hydroxybutyrate in healthy women during the last 10 weeks of pregnancy Also the plasma levels were similar to those previously found in non pregnant subjects (26) Persson & Lunell (37) have suggested that the plasma from pregnant women contains some additional titrable acid component which is not present in plasma from non pregnant women and which interferes with the titrimetric methods for FFA determination used in the studies cited above

Grumbach et al (19) showed an increase of FFA levels in hypopituitary nonpregnant individuals after an injection of human chorionic somatomammotrophin (HCS) and they have suggested that this is the pregnancy hormone responsible for the diabetogenic effects of pregnancy such as an increased insulin resistance and an enhanced lipid mobilization (20) In vitro and in vivo studies on isolated adipose tissue though have shown a more complex action of the HCS including also

an interaction between this hormone and the sympathetic nervous system (18) No increase in FFA release was seen in these studies after HCS infusion into denervated adipose tissue Also Beck & Daughaday (2) found no increase of FFA in healthy non pregnant subjects during an infusion of HCS in spite of a registered decrease in glucose tolerance Emerson et al (14) have reported that the non protein respiratory quotient remains normal during pregnancy which indicates that the metabolic effects of pregnancy are well compensated in healthy subjects

During the glucose infusion (GIT) a constantly elevated blood glucose level produces an immediate release of stored insulin The increased insulin level is sustained during the period of infusion (8) The lipolysis and the lipid mobilization as reflected in the plasma levels of glycerol and FFA are successively suppressed during the infusion (Fig 1) The LR in this study had a lower *immediate* insulin response than the HR throughout pregnancy (11) but the insulin increase at 40–60 min was similar in both groups (Fig 2) The rate and the extent of the suppression of lipolysis and lipid mobilization were the same on all test occasions as opposed to the marked increase in insulin levels during GIT in pregnancy This indicates that the diabetogenic effect of pregnancy was well compensated for both in low and in high responders

The fasting levels of FFA glycerol and D-$\beta$ hydroxybutyrate were similar in the two groups studied Nordlander et al (30) also reported fasting levels of FFA and glycerol for low responders that did not differ from those of normal controls or of diabetic patients in clinical remission The low responders as well as the diabetics showed a more pronounced increase in glycerol as well as FFA during physical exercise indicating that these subjects in certain situations do differ in their lipid metabolism

The infants as in previous investigations (33 39) showed low levels at birth of FFA and glycerol which rose considerably during the first day of life The ILR did not show any suppression of FFA levels during the first day as has been reported for IDM (9 36) The formation of ketone bodies showed a different pattern in the two groups of infants the ILR having markedly higher levels at birth and a significant *decrease* during the first day compared to the IHR

An explanation for the discrepancies in D-$\beta$ hydroxybutyrate levels at birth could be looked for in the delivery since the duration of labour has been shown to affect fetal levels of ketone bodies while fetal stress and environmental temperature seem to have less effect (35) However no correlation could be found either between the duration of labour and the D $\beta$ hydroxybutyrate level or between the birth weight and the latter The higher levels in the IHR at IVGTT could also possibly be related to a longer period of starvation in this group than in the ILR group The levels of glucose FFA and glycerol were identical before the IVGTT however and no correlation could be found between the age at IVGTT and the levels of D-$\beta$ hydroxybutyrate in ILR while this correlation was high in the IHR

In a group of infants of healthy mothers Shima et al (40) found a higher plasma level of insulin at birth among heavy infants than among those with a birth weight below 4000 grams Joassin et al (22) reported a significant positive correlation between birth weight and the insulin level at birth in a mixed group of IDM and infants to healthy mothers but apparently no correlation was found if the 5 IDM were removed Molstedt Pedersen & Jorgensen (27) demonstrated a positive correlation ($r$=0 56) between birth weight and insulin levels 3 hours after birth in their group of infants to healthy mothers but not a significant corresponding correlation in the group of infants to mothers with gestational diabetes Our findings did not confirm this postulated relationship between birth weight and insulin levels at birth or later in *normal* infants but it is also possible that the correlations found by the previous investigators reflect a relationship between birth weight and the presence or not of maternal diabetes rather than between birth and plasma insulin

In summary the women with a low insulin response showed very little or no differences from the high responders during their pregnancies regarding changes in lipolysis fat mobilization and oxidation Their infants did not postnatally show the same changes in FFA and glycerol levels as those seen in IDM but there was a difference in the D-$\beta$ hydroxybutyrate levels after birth the ILR showing a slight decrease instead of the expected increase during the fasting period This difference was not sufficiently explained by a difference in the length of the starvation period

It could possibly indicate a difference in the utilization of energy sources during this early period IDM have been shown to have an increased glycogen content in various tissues (16 32) and an active glycogenolysis (31) after birth Since the ILR in other respects have been shown to have traits similar to those of IDM such as a high glucose assimilation rate (12) they could also have larger glycogen stores than the IHR and therefore a lower rate of ketogenesis So far this is highly speculative however

## ACKNOWLEDGEMENTS

We are indebted to Dr Bertil Thalme Department of Paediatrics Karolinska Hospital Stockholm who participated in the performance of the intravenous glucose tolerance tests

This work was supported by grants from the Nordic Insulin Foundation Gentofte by the Fund in Memory of T and J Andersson Karolinska Institute Stockholm and by grants from the Swedish Medical Research Council projects No 19T-4106 and 19X 3787

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*Submitted for publication April 28 1974*

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Acta Obstet Gynecol Scand 54 357–361 1975

# THE FREQUENCY OF FETAL BRADYCARDIA DURING SELECTIVE EPIDURAL ANAESTHESIA

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*Abstract* The frequency of marked fetal bradycardia (FB) during selective lumbar epidural anaesthesia with 0 25% or 0 5% bupivacaine (Marcain® Bofors) without adrenaline in doses of 5 ml to 8 ml is reported 35 patients were monitored by means of the Hewlett Packard Cardiotocograph with Hon s spiral scalp electrode and a transcervical catheter connected to a Hewlett Packard (1 280 B/C) physiological pressure transducer Only patients with a normal cardiotocogram (CTG) before anaesthesia are included During the second stage pathological fetal heart rate (FHR) patterns and technical artefacts are common therefore the CTGs recorded in this period were not considered In the 35 patients the total time of intrauterine monitoring was 119 hours During this period 125 doses of 0 25% and 23 doses of 0 5% bupivacaine were given In 33 parturients the FHR changes observed were insignificant In one case a period of marked FB was seen One parturient developed acute hypotension with a synchronous FB while lying in the supine position It is concluded that continuous lumbar epidural anaesthesia with bupivacaine without adrenaline does not precipitate FB during the first stage when care is taken to avoid maternal hypotension

There are few and conflicting reports concerning the frequency of fetal bradycardia (FB) during epidural anaesthesia (3 7 9)

Zilianti et al (9) found a very high frequency (77%) of FB defined as a drop in heart rate below 100 beats/min during epidural anaesthesia using 2% lidocaine in doses up to 18 ml These results have been a cause of concern (3)

Marked fetal bradycardia i e heart rate below 90 beats/min with a minimum duration of two complete contraction cycles or 5 minutes is found to be associated with progressive acidosis (6) In the present paper a low frequency of marked FB episodes during selective epidural anaesthesia with 0 25% or 0 5% bupivacaine (Marcain® Bofors) without adrenaline in doses up to 8 ml is reported The purpose of this technique is to block the sensory and autonomic nerve fibres with little or no motor involvement and minimal segmental spread (1)

## MATERIAL AND METHODS

Fetal heart rate (FHR) and intrauterine pressure (IUP) were continuously recorded in 35 patients receiving lumbar epidural anaesthesia during labour Some selected characteristics are shown in Table I Only patients with a normal cardiotocogram (CTG) before blockade were included in the study Three patients were monitored because the fetuses were considered to be at risk due to prematurity (two) and to Rh immunisation (one) The other subjects represent an unselected group among the 10% of our parturients receiving continuous epidural anaesthesia during labour Among the 35 parturients of this study 30 entered the hospital in spontaneous labour Five were induced using oxytocin or prostaglandin $F_2\alpha$ and in 11 an infusion of oxytocin was started during labour There were 24 primiparae and 11 multiparae FHR and IUP were registered by means of the Hewlett Packard Cardiotocograph with Hon s spiral scalp electrode and a transcervical catheter connected to a Hewlett Packard (1280 B/C) physiological pressure transducer The monitoring was started after rupture of the membranes when the contractions had become painful usually when the cervix was dilated 2–3 cm To obtain a baseline pattern FHR and IUP were registered at least ˀ0 min before anaesthesia

The CTG s were classified according to the criteria described by O Gureck et al (6) These authors defined marked FB as FHR under 90 beats/min with a minimum duration of two complete contraction cycles or 5 min

It is well known that the number of pathologic FHR patterns increases as labour advances Therefore FHR changes during the second stage were not taken into consideration Before administration of anaesthesia and intravenous infusion of 1 000 ml Ringer s solution was started in all patients Sedatives and centrally acting analgesics were not given

The epidural block was performed with the patient in the

Table 1 *Selected characteristics of patients in study*

| | No of cases | Induced labour | Mode of delivery | | Gestational maturity (weeks) | | Apgar score | |
|---|---|---|---|---|---|---|---|---|
| | | | Spontaneous | Forceps Ventouse | <37 | 37–42 | 5–6 | 8–10 |
| Primiparae | 24 | 3 | 19 | 5 | 0 | 24 | 1 | 23 |
| Multiparae | 11 | 2 | 9 | 2 | 2 | 9 | 2 | 9 |
| Total | 35 | 5 | 28 | 7 | 2 | 33 | 3 | 32 |

left lateral position using the loss of resistance method The puncture was performed at the $L_3$–$L_4$ or the $L_4$–$L_5$ interspace using a Tuohy needle The local analgesic used was 0 25% plain bupivacaine In some cases where the effect was unsatisfactory 0 5% bupivacaine was used After a test dose of 5 ml 0 25% bupivacaine a catheter was inserted and introduced approximately 2–3 cm in the cephalic direction and connected to a Millipore Swinnex filter The patients were instructed to avoid the supine position If analgesia was not symmetrical the patients were put on the side where the pain was felt

Top up doses of 5–8 ml were given at intervals between contractions according to the needs of each patient. Maternal blood pressure was monitored with a mercury sphygmomanometer between contractions every 5 min for the first 20 min and then every 10 min Any fall of blood pressure was treated by increasing the rate of infusion of Ringer s solution

Hypotension was defined as a fall in systolic pressure low 100 mmHg in previously normotensive patients In with systolic hypertension due to pain and anxiety there is usually a fall to normal levels of blood pressure during epidural anaesthesia This was not considered to be hypotension if the blood pressure remained unchanged after delivery The upper dermatome level of analgesia to pin prick sensation was determined in every case

## RESULTS

In the 35 patients the total time of intrauterine monitoring was 119 hours During this period 125 doses of 0 25% bupivacaine and 23 doses of 0 5% bupivacaine were given The mean interval between each dose was 48 min In 33 parturients (94%) the FHR changes observed were insignificant (Fig 1) One period of FB was seen in each of 2 cases In the first subject the period of FB occurred 7 min after a top up dose of 5 ml 0 5% bupivacaine and lasted for 8 min (Fig 2) At that time the cervix was almost

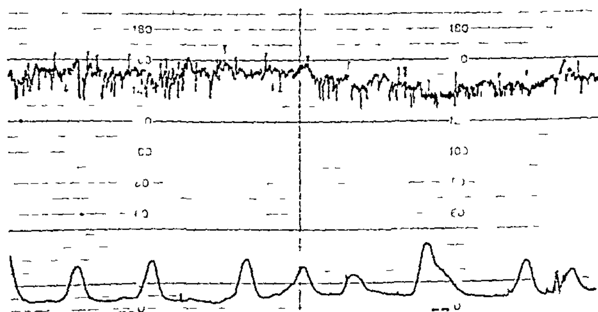
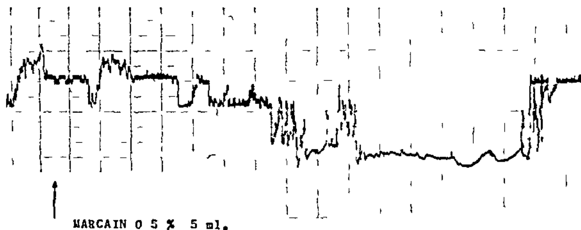


Fig 1 Normal cardiotocogram during epidural anaesthesia



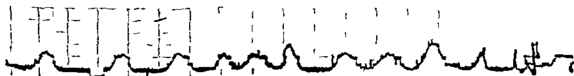




*Fig 2* Fetal bradycardia following 7 min after a top-up dose of 5 ml 0 5% bupivacaine and during a period of frequent contractions



*Fig 3* Fetal bradycardia occurring synchronously with an acute episode of maternal hypotension

fully dilated and the contractions occurred very frequently probably as a result of too rapid infusion of oxytocin

FHR returned to normal before delivery and the child had an Apgar score of 9

The second episode of bradycardia occurred during an acute fall in maternal blood pressure to 60 mmHg 17 min after a top up dose of 5 ml 0 25% bupivacaine At that moment the patient was lying in the supine position She was therefore immediately turned into the left lateral position and the infusion speed of Ringer s solution was increased Ephedrine 20 mg was given i v and oxygen was administered by face mask After about 3 min both maternal blood pressure and FHR were normal (Fig 3) The child had an Apgar score of 10 This was the only episode of maternal hypotension in the study and the very one in which a pressor agent was given

In all the subjects the upper anaesthetic level was between Th 10 and Th 7 No case of dural tap occurred

The Apgar score at 1 min ranged from 8 to 10 in 32 (91%) of the children In 3 cases the Apgar score was 5 or 6 One of these was premature and Type II dips were seen in the CTG during the second stage The second child suffered from severe erythroblastosis hich had necessitated two intrauterine blood nsfusions It was very anaemic at birth The CTG showed no pathologic changes in this case The third child was delivered by forceps due to prolonged labour The CTG was normal until the spiral electrode was disconnected

## DISCUSSION

Epidural anaesthesia may cause FB either by a direct pharmacological effect upon the fetal heart or indirectly as a result of maternal hypotension

Reynolds & Taylor (8) found a low neonatal plasma concentration of bupivacaine without any evidence of accumulation after continuous epidural anaesthesia It is unlikely therefore that bupivacaine may cause FB by a direct pharmacological effect during epidural anaesthesia It has also been shown that an intravenous infusion of bupivacaine 0 75 mg/kg in 10 min in adults does not significantly alter the heart rate (4)

Hon et al (2) has shown that maternal hypotension of less than 100 mmHg systolic pressure may be associated with FB During epidural anaesthesia in the obstetric patient an increased frequency of maternal hypotension has been reported (5) This is due to two main causes The first is the sympathetic blockade which causes widespread vasodilatation and pooling of blood in veins The second is the occlusion of the inferior vena cava which practically always occurs in the supine position During epidural anaesthesia however the compensatory vasoconstriction cannot occur and the venous capacitance is greatly enlarged Therefore an increased liability to develop the caval occlusion syndrome must be anticipated These factors were taken into consideration in order to avoid maternal hypotension in the patients studied

By using small doses of bupivacaine and injection between the contractions the extent of the block was kept below the Th 7 dermatome in every patient

All the parturients were given Ringer s solution to compensate for the vasodilatation and they were encouraged to lie on their left side in order to prevent the supine hypotensive syndrome Nevertheless one patient developed acute hypotension with a synchronous FB whilst lying in the supine position This episode lasted for 3 min (Fig 3) Due to the short duration it was not classified as a marked FB The only marked FB episode was probably a result of a too rapid oxytocin infusion followed by frequent contractions

In this study the FHR changes observed were considered insignificant in 33 of the parturients One episode of marked fetal bradycardia and one episode of maternal hypotension occurred Zilianti et al (9) reported FB in 77% and maternal hypotension in 90% of the parturients within the first 20 min after injection of 2% lidocaine in doses up to 18 ml into the epidural space In a similar study Printz & McMaster (7) found the percentages to be 11 and 10 respectively when using doses of 10–15 ml 1 5% mepivacaine The main reason for the discrepancy between the results reported here and those referred to above is probably the different dosages of local anaesthetics used It should be noted however that the three groups of parturients and the definitions of FB and maternal hypotension differs This makes direct comparison impossible

In the present study a fairly homologous group of parturients has been selected for the CTG registrations Thus all the subjects with a pathological FHR pattern before anaesthesia were excluded Due to the increased frequency of pathological FHR patterns and technical artefacts normally seen during

the second stage the CTG during this period was not taken into consideration

The definition of maternal hypotension used in this report may be more realistic than the selection of a fall in blood pressure of at least 10 mmHg within the first 20 min after epidural anaesthesia (9)

It is therefore concluded that continuous selective lumbar epidural anaesthesia with bupivacaine without adrenaline does not precipitate FB in the first stage of labour when care is taken to avoid maternal hypotension

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*Submitted for publication May 8 1974*

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Acta Obstet Gynecol Scand 54 363–367 1975

# STUDIES ON COAGULATION AND FIBRINOLYSIS IN PREGNANCY

*With Special Reference to Cold Activation of Factor VII*

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*Abstract* Studies on coagulation and fibrinolysis were performed on 40 healthy women who underwent an uncomplicated pregnancy From each subject samples were taken 6–10 times during pregnancy and all the changes were related to the subjects own 6-week postpartum values Increased concentration of coagulation factors II–VII–X was revealed by Normotest The incidence of cold activation of factor VII increased from 14% in non pregnant women in these series to 94% in pregnant women near term A marked increase in the proteolytic capacity was probably an effect of increased plasminogen concentration and decreased antifibrinolytic activity The latter did not however parallel the immunological determination of the antiplasmins The concentrations of antithrombin III and C1 inactivator of complement both decreased during pregnancy It is concluded that the overall effect of these changes is probably in favour of coagulation

The incidence of thromboembolic disease is probably increased during pregnancy (23 27) and further studies on the changes that occur in the blood might contribute to a better understanding of the mechanism behind this

The aim of this work was to record the changes in some of the coagulation and fibrinolysis factors that occur during pregnancy and also to observe when these changes appear Special attention has been paid to the recently described cold activation of factor VII (8) because it reflects an example of interference between different enzyme systems the plasma kallikrein and coagulation systems Thus a change in the incidence of cold activation of factor VII presumably indicates a change in the plasma kallikrein–kinin system

## MATERIALS

Blood was obtained at monthly intervals from 40 healthy women throughout their uncomplicated pregnancies and once 6–8 weeks after delivery Plasma was prepared as described elsewhere (8)

Normotest reagent Nyegaard & Co Oslo Thrombotest reagent Nyegaard & Co Oslo Casein Hammarsten Merck Darmstadt BRD N ethyl urethane NEU Fluka A G Switzerland Urokinase Leo Pharmaceutical Company Copenhagen Plasminogen Human Grade A AB Kabi Stockholm Antisera against $\alpha$ antitrypsin $\alpha_2$-macroglobulin and antithrombin III were prepared by immunisation of rabbits Antiserum against C1 inactivator Behring Werke Company Marburg BRD

## METHODS

Normotest (6) Thrombotest (25) Cold activation of factor VII (8) Plasminogen/plasmin caseinolysis with NEU (16) Proteolytic capacity (17) Plasminogen proactivator (?) (In the plasminogen proactivator assay the 6-week post partum values were taken to represent 100% activity ) Antiplasmin activity (16) $\alpha_1$-antitrypsin $\alpha_2$-macroglobulin antithrombin III and C1 inactivator (??) the values being expressed in per cent of those in a mixture of equal volumes of sera from 500 consecutive blood donors Since part of the antithrombin III is consumed during coagulation in vitro the normal level of antithrombin III in citrated plasma is 135% of that in normal serum Platelet count (3)

In the evaluation of the various changes every subject served as her own control the changes induced by pregnancy being presumed to return to normal 6–8 weeks after delivery

Statistical significance was evaluated with Student *t* test for paired comparison

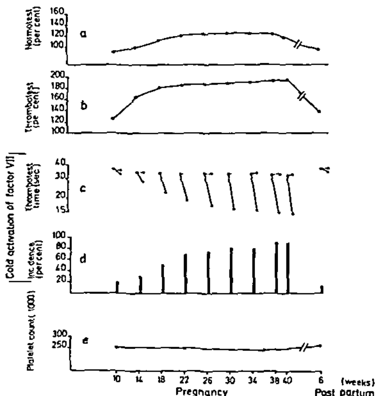



*Fig 1* The extrinsic coagulation system and platelet count in pregnancy (*a*) Normotest per cent activity in blood (mean values) (*b*) Thrombotest per cent activity in blood (mean values) (*c*) Cold activation of factor VII as revealed by the thrombotest time Immediately Left points After 20 hours incubation at 20°C — and after 20 hours incubation at 0°C — (mean values) (log scale) (*d*) Incidence of cold activation of factor VII as revealed by the percentage of the whole series that showed thrombotest times shorter than 20 seconds after 20 hours incubation of plasma at 0°C (*e*) Platelet count (mean values)

## RESULTS

### *Coagulation*

Normotest which is a screening test for the coagulation factors II–VII–X revealed increased values from the 18th pregnancy week when compared to the 6-week post partum values ($p<0.001$) (Fig 1*a*) The apparent decrease from the 38th week until term was not significant

During pregnancy an increasing number of subjects showed thrombotest activities outside the range of the standard curve For illustration purposes these were taken to represent 200% activity (Fig 1*b*) Figure 1*c* illustrates further the shortening of the thrombotest time that occurs when plasma from pregnant women is incubated at 0 C (—) for 20 hours the cold activation of factor VII (8) Incubation at 20°C did not affect the thrombotest time ( ) The figure shows the average values for the whole series The 0°C induced shortening of the thrombotest times started before the 14th pregnancy week ($p<0.01$) and the difference between the average thrombotest times after 20 and 0 incubation increased throughout pregnancy

When plasma was stored in siliconized or plastic tubes for 20 hours at 0 C the thrombotest seemed to show either cold activation with clotting times shorter than 20 seconds or no change In individual plasma specimens the thrombotest times could be as short as 10 seconds after cold activation indicating an extreme degree of factor VII activation In these series 14% of the subjects showed cold activation in plasma when not pregnant During pregnancy additional 80% of the plasmas changed from cold activation negative to positive so that at term this property was recorded in 94%

### *Fibrinolysis*

The plasma plasminogen concentration increased during the first half of pregnancy (Fig 2*b*) starting as early as before the 10th pregnancy week ($p<0.01$) The proteolytic capacity which reveals the caseinolytic activity on urokinase activation in the presence of the natural plasmin inhibitors (17) followed a similar pattern Also this parameter changed before the 10th pregnancy week ($p<0.01$) (Fig 2*a*)

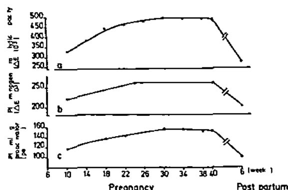



*Fig 2* Factors of the fibrinolytic system in pregnancy Changes in proteolytic capacity (*a*) plasminogen/plasmin (*b*) and plasminogen proactivator (*c*) (Mean values )

The streptokinase activated plasminogen proactivator showed a pattern that was similar to those of plasminogen and proteolytic capacity The increase was 18% before the 10th pregnancy week ($p<0.001$)

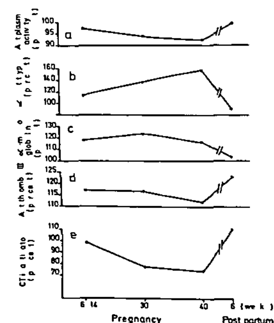



*Fig 3* Proteinase inhibitors in pregnancy (*a*) Anti plasmin activity (per cent inhibition) (*b*) Concentration of $\alpha_1$-antitrypsin (per cent) (*c*) Concentration of $\alpha_2$-macroglobulin (per cent) (*d*) Concentration of antithrombin III (per cent) The percentage refers to a standard serum normal plasma level being about 135% (*e*) Concentration of C1 inactivator of complement (per cent) (Mean values)

These changes all developed similarly whether the plasmas showed cold activation of factor VII or not

*Proteinase inhibitors*

The plasmin inhibiting activity decreased slightly during pregnancy (Fig 3*a*) The difference from the 6-week post partum value was moderate (6–7%) and statistical significance was achieved in the third trimester only ($p<0.05$)

The concentration of $\alpha_1$-antitrypsin increased throughout pregnancy The increase was about 20% as early as at 10 weeks gestation ($p<0.001$) During pregnancy only slight changes in the concentration of $\alpha_2$-macroglobulin were observed but after delivery the level decreased about 15% ($p<0.01$)

The concentration of antithrombin III decreased about 11% statistical significance ($p<0.001$) being obtained in the third trimester of pregnancy (Fig 3*d*) The concentration of C1 inactivator of complement decreased throughout pregnancy the average plasma concentration in the 1st trimester being about 10% lower ($p<0.01$) and in the 3rd trimester 37% lower ($p<0.001$) than 6 weeks post partum (Fig 3*e*)

The changes described above were not influenced whether or not the plasmas showed cold activation of factor VII

*Platelet count and haematocrit*

The platelet count changed insignificantly during pregnancy (Fig 1*e*) and the average haematocrit value showed no change that could possibly influence the assays

## DISCUSSION

Increased concentrations of coagulation factors I II V VII VIII IX and X have been observed in pregnancy while factors XI and XII probably remain grossly unchanged (19 21 24 28 31) Our results with normotest accord with the reports of increased concentration of factors II–VII–X

The most marked change that occurred in the extrinsic coagulation system was the increasing incidence of the cold activation of factor VII This phenomenon reflects the effect of plasma kallikrein upon factor VII (9) and implies an extreme degree of factor VII activation

The test is performed after 20 hours incubation

at 0°C to reduce the effect of the natural plasma inhibitors of kallikrein and other inhibitor inactivation (by acetone or low pH) causes a similar kallikrein activation of factor VII at room temperature (20) Theoretically a corresponding situation might occur in vivo because the main inhibitors of plasma kallikrein $\alpha_2$-macroglobulin and C1 inactivator of complement (13 7) both reveal broad specificities The plasma concentration of $\alpha_2$-macroglobulin shows only a slight increase in pregnancy (Fig 3c) (6) and on activation of for instance the fibrinolytic system some of the $\alpha_2$-macroglobulin will be blocked by plasmin (1) For C1 inactivator the concentration is lowered by pregnancy per se (Fig 3e) (4) and this proteinase inhibitor is also inactivated by plasmin (14) So activation of the fibrinolytic system will reduce the kallikrein neutralizing capacity of plasma and activation of other plasma proteinases might reveal a similar effect resulting in possible traces of plasma kallikrein remaining active in plasma long enough to activate factor VII

As activation of coagulation factors has been shown to be of greater importance in thrombogenesis than merely increased concentration of single coagulation factors (30) the cold activation of factor VII could indicate an *in vivo* hypercoagulable state

Our fibrinolytic assays all implied addition of an activator (urokinase or streptokinase) so nothing can be said about the spontaneous fibrinolytic activity in plasma The high concentration of plasminogen (Fig 2b) could be caused both by increased production and by a decreased consumption—as an effect of the reduced plasminogen activator activity of pregnancy (32) The increased plasminogen concentration appears to imply a variety of possible effects Plasmin presumably protects against thrombosis by the lysis of fibrin and by the fibrin degradation products revealing antithrombin activity It also attacks fibrinogen and coagulation factors VIII and V (29) Plasmin however also reveals possible procoagulant effects namely the activation of factor VII (10 12) kallikrein inhibitor inactivation as described and activation of prekallikrein (18) with the ensuing kallikrein activation of factor VII when kallikrein is not inhibited (10)

The remarkably high proteolytic capacity (Fig 2a) was probably caused by the increased concentration of plasminogen combined with a decreased inhibitor activity (Fig 3a) (15) Why the antiplasmin activity decreased when the immunological assays showed increased antiplasmin concentrations (Fig 3b c) is not clear It might be that some of the antiplasmins recorded in the immunological assays is functionally inactive

In vivo traces of thrombin and activated factor X are neutralized by the antithrombins in plasma and severe deficiency in antithrombin III (under 50% of the normal plasma level) clearly increases the incidence of thromboembolic disease (5) Though the decrease in antithrombin III concentration was slight in pregnancy (Fig 3d) it could be of importance in view of the increased coagulation potential at this time

The inter relationship between the plasma components participating in thrombogenesis is extremely complicated The exact meaning of the changes in these parameters cannot therefore be evaluated separately Together the changes described appear to increase the procoagulant potential of plasma in pregnancy

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*Subm tt d for publ cation May 13 1974*

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# BOOKS RECEIVED

*Recent Results in Cancer Research* Epithelial Abnormalities of the Cervix Uteri by F A Langley and A C Crompton Vol 40 50 Tab VIII 203 pages 1973 Cloth DM 58 – US $26 10 Berlin–Heidelberg–New York Springer Verlag

A well illustrated modern textbook including embryology normal histology epithelial abnormalities and diagnosis of malign lesions of the cervix uteri A special chapter is also dealing with the etiology of epithelial abnormalities of the cervix uteri The book is recommended to oncologists and gynecologists

*The Abortion Experience* Psychological and Medical Impact Editors Howard J Osofsky and Joy D Osofsky 35 illustrations 688 pages Price $25 00 Harper & Row Publishers Inc 1973

The liberalization and legalization of abortion in several states in the USA especially New York has given a great deal of experience and information which is presented in this book It is recommended to all interested in the program of legal abortion

*Reproduction in Man and his Ancestors for 700 Million Years* by Richard Torpin Mogowen Printing Co Augusta 1974 212 pages Price unknown

Survey of literature

*Handbuch der experimentellen Pharmakologie Androgene II und Anti androgene* Heffter Heubner/New Series Springer Verlag Berlin 1974 The book is also published in English 179 Figs 675 pages Price unknown

A very complete handbook with chapters on current views on androgen preceptors and the mechanism of androgen action determination of androgens by competitive protein binding method and radio-immunoassay determination of $C_{19}$ steroids biological determination of androgens clinic of androgens antiandrogens and their clinical use The book is recommended to endocrinologists and research workers in reproductive physiology as well as in biochemistry and pharmacology

*Ultrasonoscopic Differential Diagnosis in Obstetrics and Gynecology* by R O Meudt and M Hinselmann Springer Verlag Berlin–Heidelberg–New York 1975 199 figs 140 pages

An atlas with illustrations of good quality Text in English German French Spanish and Italian The book is of value to all using ultrasound examinations for diagnostic purpose in obstetrics and gynecology

*Respiratory Gas Exchange and Blood Flow in the Placenta* Editors Lawrence D Longo and Heinz Bartels U S Department of Health Education and Welfare National Institutes of Health Bethesda Maryland 1972 570 pp Price $6 05

*Sub-Fertility and Infertility in Africa* edited by B Kwaku Adadevoh University of Ibadan Nigeria 114 pp Price $7 50 £3 0

A summary of the results obtained at the workshop held in Ibadan Nigeria and short abstracts of the individual papers

*The Human Fetal and Neonatal Circulation* by S Zoe Walsh W W Meyer and John Lind Charles C Thomas Publisher Springfield Illinois USA 1974 Price $15 00

The book is a condensate of important research work carried out at the Department of Pediatrics at the Karolinska Hospital and the Wenner Gren Cardio Vasculary Research Laboratory Norrtull s Hospital It is well illustrated and well written and the best monography concerning perinatal circulation available at present It is recommended to post graduate students in obstetrics and pediatrics

*Maturation of Fetal Body Systems* report of a WHO Scientific Group World Health Organization Technical Report Series No 540 Geneva 1974 33 pages Price Sw Frcs 5 –

The report gives a good elementary review concerning fetal maturation and its clinical importance

*Birth Defects and Fetal Development* Endocrine and Metabolic Factors by Kamran S Moghissi Charles Thomas Publisher Springfield Illinois USA 1974 Price $24 50

This volume presents the proceedings of the VII Harold C Mack Symposium November 1971

Metabolic and nutritional factors affecting fetal growth and development as well as endocrine and environmental factors are dealt with One section is devoted to the diagnosis prevention and management of birth defects The book is recommended to all obstetricians and perinatologists

*Health Education Theory and Practice in Cancer Control* A collection of original papers UICC Technical Report Series *volume 10 Geneva 1974 105 pages*

Individual copies are available free of charge from the Managing Editor of the International Union Against Cancer 3 rue du Conseil-Général 1205 Genève Switzerland

*Colpopoiesis from the Colon* by M Kun Akademia Kiadó Budapest 1975 172 pages 122 figs Price $11 00

A very complete survey of the literature and a presentation of the authors own material of 39 patients suffering from a congenital absence of vagina The book is well illustrated and everybody dealing with such cases can learn something from the authors presentation

*Recent Progress in Obstetrics and Gynaecology* Editors L S Persianinov T V Chervakova and J Presl Excerpta Medica Amsterdam 1974 620 pages Price Dfl 170 00 US $65 50

The papers read by invitation at the VII World Congress of Obstetrics and Gynaecology are presented in this book dealing with uterine contractility biochemistry of amniotic fluid the role of hormones in tumours of the female and gynecology in adolescents The illustrations are of a high quality and the book can be recommended to all gynaecologists *A I S*

Acta Obstet Gynecol Scand 54 369–372 1975

# ANTIBODIES TO HERPESVIRUS HOMINIS TYPES 1 AND 2 AMONG WOMEN WITH NEOPLASTIC CHANGE OF UTERINE CERVIX

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*Abstract* The occurrence of antibodies to herpesvirus hominis types 1 and 2 was studied in Finland among patients with a neoplastic change of the uterine cervix The following groups were studied 270 patients with malignant or premalignant lesions of the uterine cervix 143 control patients matched for age socioeconomic level marital status home district and number of deliveries and 87 patients with breast or stomach cancer Antibodies to HVH types 1 and 2 were determined using the neutralization test Sera showing an index of ≤100 were accepted as positive to type 1 and those of ≥85 as positive to type 2 Among patients with a neoplastic change of the uterine cervix the frequency of type 1 antibodies varied between 70% and 79% In the control group the frequency was 84% and among patients with some other malignancy 91% These differences in the frequencies of type 1 antibody were not statistically significant Type 2 antibodies were present in 35 to 47% among dysplasia and malignancy of the cervix In the control group it was 18% and in other malignancies 20% The differences between these groups and the controls were almost significant significant and highly significant respectively Differences could also be observed in the distribution of neutralization indexes The average index calculated from all sera was 48 both in the control group and in the group of other malignancies while in sera of patients with dysplasia carcinoma in situ and invasive carcinoma the respective values were 70 66 and 79

The results are discussed in terms of the role of herpesvirus hominis type 2 in the development of cervical cancer

Carcinoma of the uterine cervix is very seldom found in childhood A few cases have been described (3 5) all of those being adenocarcinoma however

There is evidence suggesting that in some cases cancer develops gradually starting with mild atypia of cells Epithelial changes progress through dysplasia to carcinoma in situ and ultimately to invasive carcinoma The average intervals between the different stages are unknown but suspected to be several years (10)

Despite the large body of data available the etiology of cervical cancer is obscure The risk of acquiring the disease appears to be related to hygiene and to way of life Cervical cancer is very seldom found among nuns and relatively seldom among Jewish women Among Negroes and prostitutes the disease is not uncommon Epidemiologic studies have shown that women with cervical cancer indulged in heterosexual activities earlier in life and tended to have more sex partners This then raises the question of what may be the carcinogenic factors related to promiscuity which initiate the atypical change in the cervical epithelium

Genital herpesvirus infection is agreed to be a venereal disease In a previous study it was demonstrated that HVH type 1 antibodies was present already in children s sera whereas HVH type 2 antibodies could not be detected until age 14 years (15)

The possibility that herpesvirus type 2 is an oncogenic agent in squamous cell carcinoma of the uterine cervix is suggested by the parallelism between this malignancy and herpes genitalis Sero-epidemiologic data since 1968 (18 16 17 13 20 1 4 15) and also recent antigenic studies (7 19) strengthen the suspicion that the virus may play a carcinogenic or cocarcinogenic role

The present study was made to obtain information on the frequency of antibodies to herpesvirus hominis types 1 and 2 in sera of patients with squamous cell malignancy of the uterine ...

Table I. *Distribution of the study population into various groups. Number and mean ages of patients in these groups*

| Group | Number of patients | | Mean age |
|---|---|---|---|
| | in subgroups | in groups | |
| Patients with cervical lesion | | | |
| Dysplasia | | 61 | 36.0 |
| Carcinoma in situ | | 52 | 44.0 |
| Infiltrative carcinoma | | 107 | 53.9 |
| stage Ia | 14 | | |
| stage Ib | 26 | | |
| stage II | 27 | | |
| stage III–IV | 40 | | |
| Control patients | | 143 | 44.9 |
| Patients with some other type of cancer | | 82 | 60.3 |
| ca mammae | 51 | | |
| ca ventriculi | 31 | | |

## MATERIAL AND METHODS

*Study groups.* The patients were detected by mass screenings performed in the southwest of Finland. Those with histologically confirmed cervical anaplasia were admitted to the Department of Obstetrics and Gynecology, University of Turku. A few of the patients were sent by rural or urban medical practitioners. The nomenclature used is that suggested by the International Federation of Gynecology and Obstetrics in Vienna 1961. The matched control patients were selected retrospectively according to the following criteria: age, socioeconomic level, home district, marital status and number of deliveries. These controls had no herpetic manifestations, no cytological evidence of cervical anaplasia and no cancers. The other control group consisted of females with breast or stomach cancer. In each case the diagnosis was confirmed histologically. The distribution of patients into different groups is shown in Table I.

Laboratory methods have been described in the previous report (15).

Antibodies to HVH types 1 and 2 were determined with the neutralization test. The degree of neutralization was expressed as an index. Sera showing an index of $\leq 100$ were accepted as positive to type 1, and those of 85 or more as positive to type 2 as well.

The degree of statistical significance of differences was determined by Student's *t* test, the chi square test and variance analysis. The difference between two values is said to be almost significant if $0.01 < p \leq 0.05$, significant if $0.001 < p \leq 0.01$, and highly significant if $p \leq 0.001$.

## RESULTS

The frequency of antibodies in the different groups is shown in Table II. Of 143 specimens of the matched control group, 26 had a neutralization index of $\geq 85$ when tested with type 2 virus (18 %). The carcinoma in situ group had a rate of type 2 neutralizing antibodies of 35 %, the difference between this and the rate of the control group being almost significant. Among the 61 patients with dysplasia, 38 % were positive, whereas 47 % of the patients with invasive carcinoma were positive, the differences between these two study groups and the controls were statistically significant and highly significant respectively. Because the frequencies in the breast cancer (18 %) and stomach cancer (23 %) groups did not differ significantly from the control group, these subgroups were combined. The rate of positive reactions among patients with other malignant diseases was then 20 %.

With type 1 virus, no significant differences were found between any of the groups. The frequencies were 70 to 91 % and in the control group the rate (84 %) was much higher than the rate of type 2 antibodies (18 %).

The degree of neutralization was expressed as *K* values as described earlier. Average values of $K_1$, $K_2$, standard deviations and neutralization indexes ($NK_2$) of each group are shown in Table III.

The $NK_2$ value was the same, 48, in the control

Table II. *Frequency of neutralizing antibodies to herpesvirus in cancerous or precancerous groups*

| Group | Number of patients | | | Compared with the control group |
|---|---|---|---|---|
| | Tested | Positive type 1 | Positive type 2 | |
| Dysplasia | 61 | 44 (72 %) | 23 (38 %) | <0.01 |
| Carcinoma in situ | 52 | 41 (79 %) | 18 (35 %) | <0.05 |
| Infiltrative carcinoma | 107 | 75 (70 %) | 50 (47 %) | <0.001 |
| Control | 143 | 121 (84 %) | 26 (18 %) | |
| Other malignancies | 82 | 75 (91 %) | 16 (20 %) | <0.5 |
| ca mammae | 51 | 45 (88 %) | 9 (18 %) | |
| ca ventriculi | 31 | 30 (97 %) | 7 (23 %) | |

Table III *K values of sera tested to HVH type 1 and type 2*

| Group | $K_1$ | S D | $K_2$ | S D | $NK_2$ | *P* Compared with the control group |
|---|---|---|---|---|---|---|
| Dysplasia | 2 98 | 2 06 | 2 11 | 1 65 | 70 | <0 01 |
| Ca in situ | 3 41 | 1 47 | 2 25 | 1 38 | 66 | <0 01 |
| Invasive ca | 3 70 | 1 78 | 2 97 | 1 75 | 79 | <0 001 |
| Control | 2 61 | 1 65 | 1 24 | 1 05 | 48 | |
| Other malignancies | 2 96 | 1 65 | 1 45 | 1.35 | 48 | |

group and in the group of patients with other malignancies In statistical analysis of $NK_2$ values by the chi square test sera of patients with dysplasia or carcinoma in situ showed significantly higher $NK_2$ values than sera of the control group In *the infiltrative carcinoma group the average value* was highly significantly greater 79

## DISCUSSION

Highly varying values have been published for the frequency of HVH type 2 antibodies in patients with cancerous or precancerous cervical lesions (18 16 17 13 20 1) In the present study the frequencies are comparatively low Discrepancies in results may be due to differences in selection of patients Results of different studies are also rendered incomparable by the fact that the serologic methods employed have differed Furthermore there are inconsistencies in the expressions of the titres and in the methods of comparison The analysis of the results is also hampered by the variety in the *terminology used for the classification of* epithelial atypias of the portio Adam et al (1) included sexual history in their control criteria They observed no statistically significant differences in antibody rates between patients with cervical cancer and their matched controls In their discussion these workers describe their control series as a population sub group likely to run a high risk of subsequent cervical cancer

The significantly greater occurrence of HVH type 2 antibodies in females with cancerous or precancerous lesions of the cervix is not enough to substantiate a causal relationship Besides cell transformation this *association observed* by several investigators (18 17 13 4 20) may signify herpetic infections before simultaneous with or after neoplastic changes

Various hypotheses can be suggested For example during the transformation the nucleic acid of the virus or part of it may associate with the genetic material of the cell itself and cause an immediate malignant growth (direct change) When herpetic infections occur prior to neoplastic changes part of *the nucleic acid of the virus may be retained in the* cell without any change in the growth of cell External factors however may lead to subsequent transformations (indirect change) Such precipitative factors may be hormonal metabolic or *irritative* factors or any traumas ensuing from delivery (15) Alternatively a common cause may promote both herpetic infection and neoplastic changes without any causal relationship whatsoever In certain age groups e g at puberty the epithelium of the portio may be more susceptible to virus infections than at other times After a primary infection HVH type 2 may then be retained in latent form Carcinogens may subsequently activate the virus although this does not necessarily cause any transformation

HVH type 2 may also enter the organism *simultaneously with a carcinogen* and then result in infection Neoplastic changes may even precede herpetic infection In this case the cellular tissue may have become less resistant the virus infection being merely a secondary phenomenon Numerous studies of the pathogenesis of the carcinoma of the uterine cervix have been conducted over the past few years and several factors have been shown to be related to these lesions (9 2 6 8 11 17 12) It seems highly doubtful that a single etiologic agent could induce this malignancy The present study also seems to suggest several responsible agents HVH type 2 acting however as one of the pre*cipitating factors*

Cervical carcinomas are more frequent than other malignant tumours in the younger age groups Average recovery in treated cases is approximately 50 % but considerably *higher percentages (even 90)*

of recovery can be reached when the lesions are detected early (?1 14) Better insight into the path ogenesis of the disease will permit earlier detec tion and thus lead to a better prognosis The follow ing studies will show if the prognosis could be further improved by tracing persons with HVH type 2 antibodies because they are likely to form a high risk group with regard to cervical cancer

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*Submitted for publication Aug 15 1974*

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Acta Obstet Gynecol Scand 54 373-384 1975

# THE USE OF ELECTRO ANALGESIA IN OBSTETRICS AND GYNECOLOGY

*A Survey by Invitation*

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*Abstract* The newly developed method of electroanalgesia is based on the employment of low intensity impulse currents with extended frequency range and electrode application in the region of the forehead and mastoid processes Electro analgesia has been successfully used in preparing the pregnant woman for labour in controlling abnormal uterine contractility in preventing and treating toxaemia of pregnancy It produces favourable effects on the mother and the fetus Electro-analgesia has also been successfully used in labour anaesthesia and as part of a combined method of general anaesthesia in 200 obstetrical and gynaecologic operations and the post operative period

The possible unfavourable influence of psychological factors on the physiological functions of the body on the process of labour and the pattern of uterine contractility is universally admitted

Pathologic and painful labour a forthcoming operation the anticipation of postoperative pain—all these can arouse in the patient a feeling of anxiety and fear The thought of pain the possibility of pathologic labour the danger of an operation the dread of narcosis and also emotions associated with a probable loss of vital organs and its impact on the menstrual function child bearing ability family life arouse negative emotions more or less expressed

In a woman s life emotions accompany great joy painful doubts and disillusionment

Emotions are an objectively existing natural phenomenon they accompany us all through our lives helping us to assess the utility or harm of any present situations both inner and outer in the quickest possible way (P K Anokhin 1966)

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In the Soviet Union all women are guaranteed expert and free medical assistance in labour the pregnant woman and her fetus are closely monitored throughout pregnancy to prevent complications or reveal their early signs and provide timely arrest of their development

The method of physio psycho prophylactic preparation of the pregnant woman for labour developed in the Soviet Union has received wide recognition both in our country and abroad Analgesia in labour and obstetrical operations is achieved by a variety of pharmacological means

However the pharmacological analgesia of labour and obstetrical operations (caesarean section etc ) poses a problem in solving which one has to consider possible negative effects on the mother as well as the fetus and the newborn

Those means which neither negatively affect the maternal and fetal systems nor interfere with uterine contractility but yet prevent abnormal development of labour or atonic and hypotonic bleedings which constitute a threat to the life and wellbeing of a woman seem the most expedient

Despite a massive research effort in this direction despite comprehensive clinical data accumulated despite a number of unmistakable achievements the problem of new means of pharmacological labour analgesia and contractility control is still far from solved

The present situation is aggravated by an increasing incidence of allergic responses and idiosyncratic reactions to some anaesthetic drugs of general and local action

Proceeding from the above considerations one can fully support the search for new ways capable of suppressing negative emotions elevating the

of recovery can be reached when the lesions are detected early (21 14) Better insight into the path ogenesis of the disease will permit earlier detec tion and thus lead to a better prognosis The follow ing studies will show if the prognosis could be further improved by tracing persons with HVH type 2 antibodies because they are likely to form a high risk group with regard to cervical cancer

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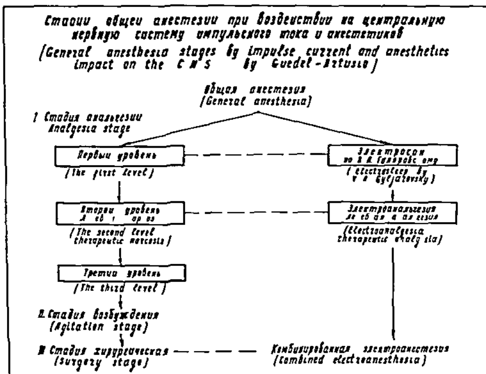



*Fig 2* General anaesthesia stage achieved through the effects of impulse currents and anaesthetics on the central nervous system (by Guedel Artusio)

anaesthesia appears to be the optimal depth of anaesthesia required for therapeutic purposes (Fig 2)

The data reported by Artusio (1954) Lukich (1965) Petrovsky & Efuni (1967) and others demonstrate that the analgesia stage is characterized by minimal reflex activity combined with stable pulse respiration and arterial pressure indices This should be looked upon as evidence of stable vegetative balance

In every-day obstetrical practice we can often observe how pain sensations negative emotions fear of possible complications for the mother and the infant are accompanied by a slackening of the auto-regulation mechanisms in the central nervous system and consequent disordered vegetative balance Not only are pulse and respiration indices disordered against this background but abnormal labour (discoordination etc ) develops as well In such instances therapeutic obstetrical narcosis in conjunction with various pharmacological means has been successfully employed for quite a time

Therefore everything stated above provides a physiological substantiation for therapeutic analgesia being used to regulate vital bodily functions and the labour process

The criterion by which to assess the optimal level of electro-narcosis was furnished by the appearance of clinical symptoms characteristic of the second level in the first stage of general anaesthesia Here we have noted a stabilization of pulse respiration and blood pressure indices even in the presence of throbbing pain The study of the brain cortex electrical activity has brought out the similarity in the development of general anaesthesia stages induced both with impulse currents and pharmacologic agents (EEG findings Fig 3) Polycardiographic and electrocardiographic findings do not show the electro-narcosis to produce any harmful effects on the heart muscle performance—moreover it normalizes the cardiac rhythm The findings of rheohysterography and phase analysis of the cardiac activity testified to a better status of the fetus and the newborn which might be associated with controlled labour activities and normalised uterine-placental circulation

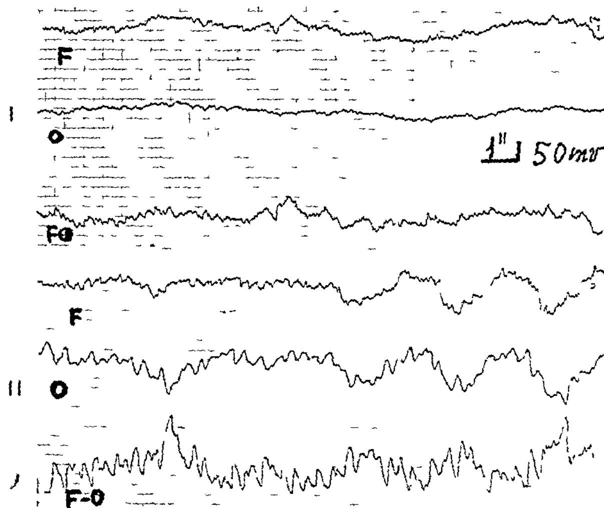



*Fig 3* I The EEG of active waking prior to analgesia administration II The EEG of inhibition state with the hypothalamic synchronizing system activated

*Our research and clinical observations have made* it possible for us to develop a new method of preparing pregnant women for labour in conditions of the in patient clinic and out patient obstetrical consulting centre (E M Kastrubin) [1] This method of preparing for labour with the aid of impulse current analgesia is intended for pregnant women with aggravated obstetrical or gynaecological anamnesis somatic diseases or late toxaemia As a result of this preparation the signs of toxaemia diminished blood supply to the brain was stimulated the chance of abnormal labour development was prevented the duration of labour shortened uterine vascular tone and hyperaemia increased fetal cardiac activity also improved Both the postpartum and the neonatal period took a favourable course The functional parameters of the central nervous system in the postpartum did not differ from those observed in the group of parturients with normal labour (Persianinov & Kastrubin 1971)

We have staged the method of electro analgesic preparation for labour and electro analgesic prevention and treatment of late toxaemia (Safronova & Chistiakova) in the in patient clinic of our Institute and in Women s Consulting Centre No 12 Moscow

Our experience has indicated that electro-analgesia can be successfully used to prevent severe forms of late toxaemia in cases where the risk of such a complication arises (extragenital diseases

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increased excitability vascular tone and fat metabolism disorders aggravated obstetrical anamnesis etc ) in the in patient and out patient clinic alike

Electro analgesia has proved to be an effective method of treatment of the early stage of late tox aemia and in mild nephropathy In cases of ad vanced nephropathy this treatment is effective in combination with other therapeutic measures

EEG findings demonstrated normalizing the electrical activity of the brain under the impact of electro-analgesia The action of the impulse cur rents was accompanied by a vasodilating effect as evidenced by study of the vascular tone and hyperaemia of the brain

When electro analgesia was performed to treat signs of late toxaemia clinical manifestations of fetal hypoxia were diminished

The idea of using electro analgesia to control abnormal labour activity is based on the concepts of physiological mechanisms underlying uterine con tractility regulation

The act of labour occurs in the presence of the formed labour dominant (Yakovlev 1957 Garmasheva 1952 and others) which incorporates higher nervous centres and executive organs into one dynamic system where reflexes providing optimal conditions for the progress of labour gradu ally prevail while other reflexes which at that time are of little consequence are suppressed

Simultaneous involvement of cerebral rhythms and various vegetative functions in response to trig ger stimulation suggests that the nucleus of the functional system controlling the labour process (labour dominant) is located in the deep structures of the brain—hypothalamus and the limbic system (Lebedeva & Orlov 1969)

The principal role in the neurohumoral regulation of the uterine function and labour contraction in cluded in the intact body belongs to the hypo thalamus and limbic complex structures and in the first place to almond shaped nuclei and cortical formations in the temporal lobes of cerebral hemispheres (Kullanda 1958 Orlov 1961 1963 Usoskin 1968 Lebedeva & Orlov 1969 and oth ers) The central nervous system exercises higher and fine control of the labour process

The fact of myogenous uterine excitability does not exclude the controlling role of the central nerv ous system the afferent connection of the uterus with the central nervous system underlying the re flex control of its activity The afferent link of regu lation comprises the pituitary and hypothalamic hormonopoeisis function manifested in an increased secretion of oxytocin and gonadotropic hormones in response to excitation of the uterine receptory system (Nikolaev 1951 Lisogor 1958 Yakovlev 1965 and others)

Normal uterine contractility in labour is characterized by the presence of the triple de scending gradient where the contraction wave is initiated in the region of the uterine bottom and spreads to the right and to the left diminishing in intensity and duration while descending

Any deviation from the triple descending gra dient principle is accompanied by a manifestation of discoordinated uterine contractility

It is a well known fact that painful labour can lead to uterine contractility disorders especially so when it is coupled with negative emotions The development of discoordinated labour activity is often associated with the fear of labour conflicts of daily routine the state of anxiety and hypochondria during labour

Usoskin (1974) proceeds from his experience with patients suffering from diseases of the central nervous system to note that the highest incidence of complicated pregnancy and labour (uterine inertia toxaemia of pregnancy etc ) is observed in cases of pathologic changes in the diencephalic and hypo- thalamic region when the pathologic process is localized in the limbic system

Snabanach et al (1964) reported that disordered uterine contractions (gradient inversion uterine hypertone asynchronous contractions) occur most commonly in cases of disordered balance between the sympathic and parasympathic links of the veg etative nervous system

According to Gellhorn and Loefbourrow (1966) emotional sensations emotional expression out ward behaviour and changes in inner organs are all results of certain types of activity of the central nervous system These authors present a vast ac cumulation of data to substantiate their opinion that the limbic system plays a prominent role in the formation of emotions specifically the role of mediator between the hypothalamus and the cere bral hemisphere cortex thus determining the qual ity of the emotional state which is experienced on the level of the cerebral cortex The hypothalamo- limbic system along with the reticular formation and the new cortex comprise the major mechanism of

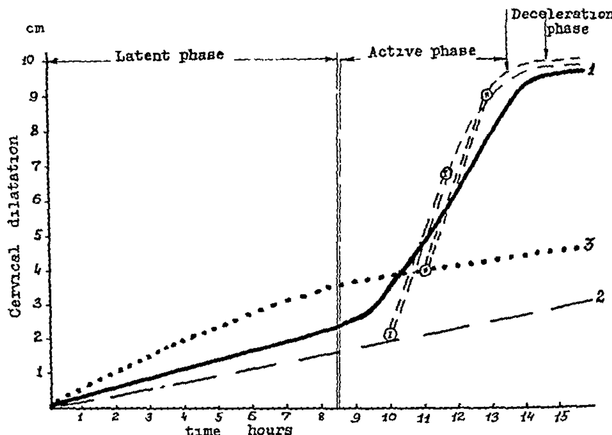



*Fig 4* The effect of electro-analgesia on the speed of cervical dilatation in various labour phases in comparison with the normal course of labour (1) Normal labour (from E Friedman) (2) decelerating labour process in latent phase (3) decelerating labour process in active phase Interval electro analgesia

the central nervous substrate of emotions The hypothalamo-limbic system is of paramount importance for emotional sensations and emotional expression The excitation of hypothalamus enhances cortical activity and vice versa When the hypothalamic excitability decreases (as in the case of posterior hypothalamic lesion) the excitation of the cortex fades away Brady (1960) pointed to a specific role of the cortical frontal lobes inhibiting the hypothalamus Shifts related to cortical involvement should be considered from the viewpoint of integral relations between the limbic system and diencephalo-cortical interactions

According to Anokhin (1966) emotions embrace the entire organism they lend a certain biologic quality to the status of an individual Integrating in a synthetic whole all the bodily functions almost instantaneously emotions as such can be an absolute signal of a beneficial or harmful impact on the organism

Vegetative shifts (pulse arterial pressure intestinal peristalsis) reflect the emotional state in a more precise way than do skeletal muscle reactions

Correlations between sympathetic and parasympathetic reactions in various types of emotional expression are of great significance Gellhorn & Loefbourrow (1966) are inclined to believe that emotions of two opposite biological values—biologically negative (pain fear anguish) and biologically positive (satisfaction) ones—have different chemical substrata underlying their expression and are characterized by different levels of involvement of cholinergic and adrenergic mechanisms Similar opinions have been put forward by other scientists as well

A vast amount of data reported on the subject points to the necessity of a multilateral approach to the assessment of hypothalamic effects in the study of mechanisms underlying activities and emotions both in normal and in pathological conditions Parasympathic reactions are initiated on excitation of the anterior hypothalamic region while sympa

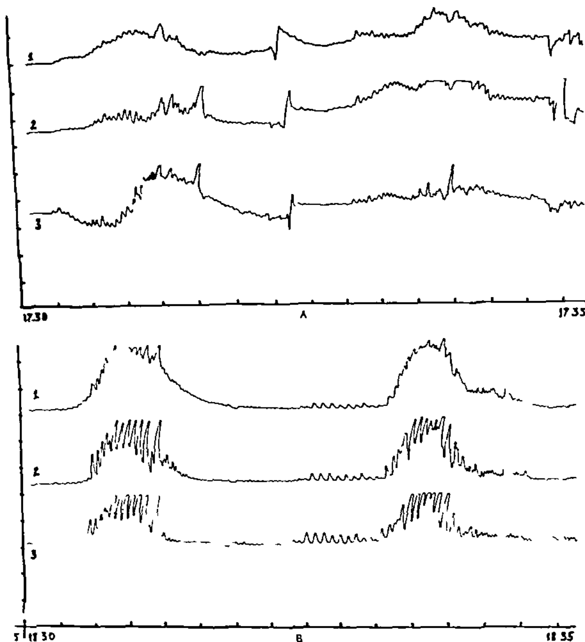



*Fig 5* Uterine contractility of parturient P (A) A hysterogram prior to an electro-analgetic session shows discoordination with lower segment hypertone (B) In the course of electro-analgesia labour forces have normalized to exhibit the triple descending gradient (1) Fundus uteri (2) corpus uteri (3) lower segment the time-course indicated below

thic reactions stem from excitation of the posterior and lateral regions

Hypothalamic excitation or emotional excitement leads to a powerful discharge of the entire sympathic system accompanied by a hyperfunction of the adrenal medullar substance (sympatho adrenal activation)

The hypothalamus is also known to considerably affect the activation of anterior pituitary producing trophic hormones which are specific for various

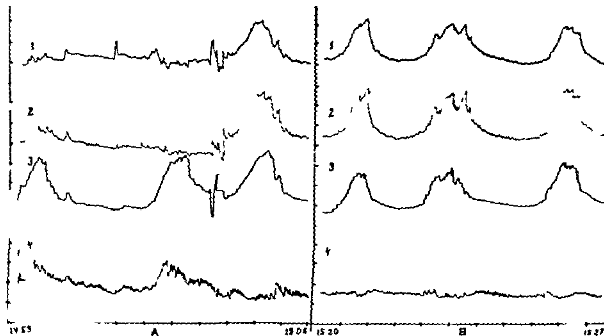



*Fig 6* Uterine contractility of parturient S (A) A Hysterogram prior to an electro analgetic session shows discoordination of contractile uterine activity (B) after electro analgesia the registration of the uterine contractions showed a normalisation of the contractile activity

internal secretory glands and stimulate thyroid and gonadal functions

Nervous and endocrine factors involved in emotions are closely connected with hypothalamic activity and via the latter with the limbic system cerebral cortex and endocrine system

The interrelations of the endocrine system with higher departments of the central nervous system based on the feed back principle are of decisive importance not only in forming adaptation reactions of the body but also in maintaining homeostasis and shaping behavioural responses (Selye 1950 Harris 1955 Horizontov Protasova 1968 Kakhana 1968)

The neuro-endocrine system is a complex system a structural basis of auto organization and autoregulation processes in this system being provided by annular connections among its individual links Nervous mechanisms are switched on when a receptor apparatus of any organic system receives an influence of any kind

The use of impulse current analgesia with electrodes placed on the forehead and neck suggests the assumption that electro analgesia affects the hypothalamus limbic complex structures which are of paramount importance for emotional sensations and expression (Gellhorn & Loefbourrow 1966) as well as cortical frontal lobes whose inhibiting action on the hypothalamus is stressed by Brady (1960)

The positive results yielded by this method in the treatment of discoordinated labour forces furnish the evidence of electro-analgesia affecting the hypothalamus and the limbic system as both are of cardinal importance in the neurohumoral regulation of labour

Vegetative shifts (pulse arterial pressure respiration etc ) arising under the influence of emotions in the case of disordered balance between the sympathic and parasympathic link of the vegetative nervous system are repaired by electro analgesia the indices of pulse arterial pressure and respiration becoming stabilized

We have used electro-analgesia to control labour activity in various types of its discoordination

We have studied clinical effectiveness rates of analgesia used for prevention (latent phase) and treatment (active phase) of protracted labour in 170 cases and in 200 cases of pronounced discoordination of uterine contractility (Kastrubin Rusina Papitashvili)

When electro analgesia was applied in the case of a prolonged preliminary stage uterine contractions were arrested in a number of patients to be re

sumed in 3–7 days as normal labour The majority of patients however in response to electro analgesia immediately developed regular contractions which resulted in spontaneous labour

An electro-analgetic session as a rule contributed to a quicker cervical dilatation (Fig 4) and the appearance of coordinated uterine contractions

The electro-analgetic effect could be most clearly seen in the treatment of discoordination manifested in an irregular contraction tone in various sections of the uterus and disordered coordination and the triple descending gradient hypertone being frequently observed in the region of the lower segment (Figs 5 and 6) or the body of the uterus

Within 20–30 minutes of an electro-analgetic session uterine contractility normalizes which is manifested in contractions prevailing in the bottom region and the presence of the triple descending gradient Labour takes a normal course fetal cardiac activity improves along with the improvement of uterine–placental circulation Signs of fetal asphyxia recorded by phono-electrocardiography and cardiotachography disappear under the effect of electroanalgesia The newborns scored 8–10 Apgar points and study of acid–base balance in the fetal blood revealed normal ratios

Not only does electro-analgesia normalize discoordinated uterine contractility it also reduces the blood loss in the placental and puerperal stages

The analgetic effect of the method in labour is obtained by means of extending the range of succession of impulses (to 750–1 000 Hz) in specialized electro-analgetic equipment developed by Nozhnikov and Kastrubin

Our experience with electro-analgesia in obstetrics allowed us to try impulse currents for surgical analgesia—first in gynecological surgery (120) and later in cesarean section (80) Here a method of combined electro narcosis (nitrous oxide plus impulse currents) has been developed (L S Persianinov N N Rasstrighin S N Dizna & E M Kastrubin A Method of Electroanaesthesia Copyright certificate No 454916 Bull No 48 1974)

Its scheme is simple and available in all conditions In gynaecologic surgery—common premedication i e 2 ml 2% Dimedrol 1 ml 0 1% Atropin administered intramuscularly 20–30 min prior to the narcosis In caesarean section premedication is limited to the intramuscular injection of 0.5–1 ml Atropin or Methacine

Inductive narcosis is achieved with intravenous

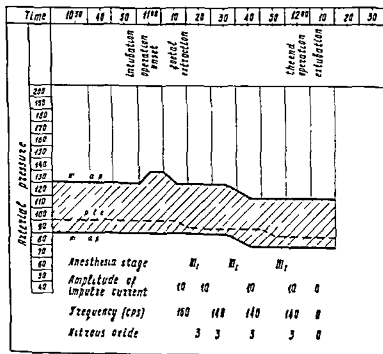



Fig 7 The indices of the systolic and diastolic arterial pressure and pulse under the action of combined electronarcosis in caesarean section (patient N )

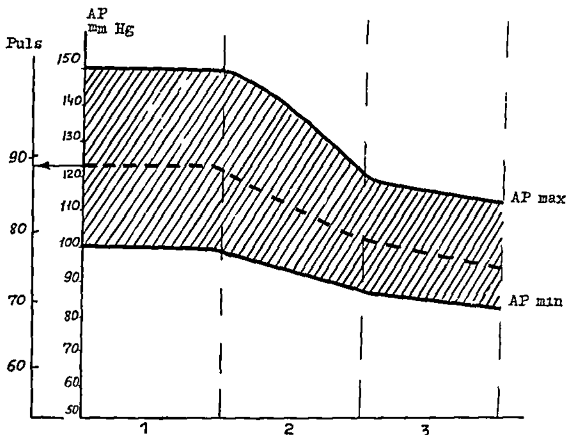



*Fig 8* The indices of arterial pressure and pulse rate ( ) during an electro analgetic session in the postoperative period

Sombrevin (Sombrevin=Epontol) a barbiturate free general anaesthetic of super short action Following intravenous administration of muscle relaxants (100–120 mg Lysthenon) tracheal intubation is performed providing artificial lung ventilation

A combination of gas mixture (60–70% nitrous oxide and 30–40% oxygen) and electro analgesia secures an adequate level of narcosis throughout the entire operation The above scheme of combined electro narcosis reduces the possibility of harmful effects on the mother the fetus and the newborn as well as on uterine contractility

According to clinical data and indirect objective tests (the indices of arterial pressure pulse ECG plethysmography acid–base values oxyhaemoglo

*Spirographic control of electroanalgesia as factor of intensive therapy*

*I Before electroanalgesia*

| | |
|---|---|
| Respiration rate | – 28 (1) |
| Respiration capacity (ml) | – 220 |
| Minute resp capacity (ml) | – 6160 |
| Consume $O_2$ (ml) | – 280 |

*II In the course electroanalgesia*

| | |
|---|---|
| Respiration rate | – 20 (1) |
| Respiration capacity (ml) | – 280 |
| Minute respiration capacity (ml) | – 5600 |
| Consume $O_2$ (ml) | – 240 |

*III After electroanalgesia*

| | |
|---|---|
| Respiration rate | – 14 (1) |
| Respiration capacity (ml) | – 300 |
| Minute respiration capacity (ml) | – 4200 |
| Consume $O_2$ (ml) | – 230 |

*Fig 9* A spirogram and external respiration indices of patient N in the postoperative period after the supravaginal hysterectomy The effect of electro-analgesia on spirographic record (1) Before electro-analgesia ( ) during electro analgesia (3) after electro-analgesia

bin) the depth of combined electro narcosis attains the optimal level ($III_1$) Muscle relaxant administration and artificial lung ventilation are performed according to common principles

In case of uncomplicated pregnancy and in the absence of intra uterine fetal distress newborns were assessed at 8–10 Apgar The combined (nitrous oxide plus impulse currents) endotracheal narcosis employed in gynaecological surgery and caesarean section alike is characterized by more stable haemodynamic indices (Fig 7)

Electro-analgesia has also been applied (Dizna Proshina) as anaesthesia during the postoperative period To achieve the electro-analgetic stage the session lasted for 60–90 minutes The postoperative patients experienced first painful sensations within 6 to 12 hours If the patient sensed pain she was given the first Promedol (Promedol=Trimeperedin) injection both single and daily doses of Promedol being 3–4 times lower here A more pronounced analgetic effect was registered

*Electro-analgesia is an effective method of reliev*ing pain in the postoperative period The use of impulse currents according to our pattern produces a regulating effect on the cardio vascular system and the respiratory function in postoperative patients Arterial pressure stabilizes (Fig 8) the external respiration and gaseous exchange function improves (Fig 9)

The impact of electro-analgesia is free of any side effects or narcotic depression the patients are more active physiological functions of the body recover sooner Our experience leads us to assume that the method of postoperative analgesia with impulse currents alone or in combination with low-dose analgetics is gaining in importance in the management of postoperative patients

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*Submitted for publication Febr 11 1975*

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Acta Obstet Gynecol Scand 54 385–386 1975

## SHORT COMMUNICATIONS

# SIMULTANEOUS URETHROCYSTOMETRY AND URETHRA PRESSURE PROFILE MEASUREMENT WITH A NEW TECHNIQUE

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*Abstract* A new technique for simultaneous recording of the pressures within the urethra and urinary bladder is described The pressures are recorded by micro-transducers enclosed in a semiflexible dacron catheter The recording system has a high frequency response (2000 Hz) and is free of motion induced artefacts which makes it possible to measure the urethra pressure profile with a high degree of precision

In spite of intensive research our knowledge about the origin of urinary incontinence is still very moderate This is most probably due to the incomplete and uncertain investigative methods which have hitherto been used Simultaneous urethrocystometry has previously been performed by fluid filled catheters connected to conventional transducers (2 4) However these systems have several disadvantages They are difficult to calibrate and they may have an inadequate frequency response Measuring artefacts are easily introduced if the apertures of the catheters are blocked by the mucosa or if an air bubble enters the recording system (1 5)

### *Present technique*

Two micro-transducers (Millar Instr Inc Houston Tex USA) are enclosed 6 cm apart in a semiflexible Dacron catheter (Fig 1) The transducers had previously been tested in a specially designed calibrator and their frequency response was found to be more than 2000 Hz (1 5) The catheter is connected to an amplifier Besides amplifying the signals from the two transducers it also amplifies the difference between these two signals The signals are recorded by an ink jet mingograph Calibration and sterilization takes place in the calibrator (1)

### *Recording procedure in vivo*

With the patient in the lithotomy position the catheter with the two transducers is introduced transurethrally into the bladder The catheter is then drawn slowly out of the urethra During this manoeuvre transducer no 2 (Fig 1) is recording the intra urethral pressure from the inner to

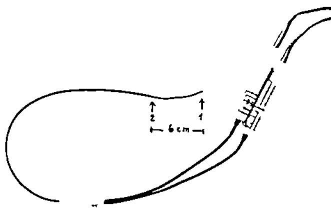



*Fig 1* Photo of the catheter with the two microtransducers enclosed The localization of the transducers is indicated by arrows The distance between no 1 and no 2 is 6 cm The outside diameter of the catheter is 2 3 mm

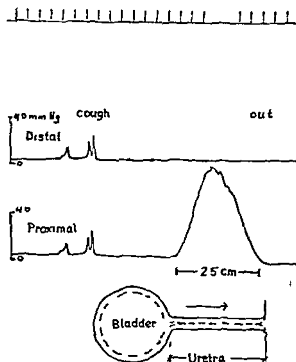



*Fig 2* Diagram of a urethra pressure profile Top curve = bladder pressure bottom curve = urethra pressure To the left both the transducers are situated in the bladder A cough indicates equal pressure transmission from both transducers The catheter is then drawn slowly back
The proximal transducer no ? is now recording intra urethral pressure from the inner to the outer
ie urethra pressure profile The distal trans ducer no 1 still in the bladder records at the same time the intravesical pressure The maximal intra urethral pressure amplitude is in this case recorded 1 1 cm from the inner meatus The functional length of the urethra is ..5 cm

the outer meatus (Fig ?) Transducer no 1 still in the bladder is recording the intravesical pressure (Fig ?)

## COMMENTS

*Over a 2 year period more than 100 patients with* various urological disorders have been examined with the present technique Most of the patients suffered from urinary incontinence The pressure diagrams in Figs 2 and 3 clearly demonstrate that with the present technique certain and detailed information on the intra urethral and intravesical pressures under different conditions is obtained A more detailed description of our construction as well as the results from the investigations of different groups of patients will be related later in this journal

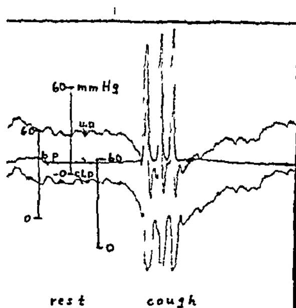



*Fig 3* Pressure diagram from a patient with moderate stress incontinence degree I according to Ingelman Sundberg (3) At rest the closure pressure i e the urethra pressure minus the bladder pressure is about 20 mmHg (bottom curve) When the patient coughs the closure pressure drops to 0 and urine now escapes the urethra

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Acta Obstet Gynecol Scand 54 387–388 1975

# INTERFERFNCE OF ANTISEPTICS CONTAINING HEXAMETHYLENE TFTRAAMINE (HIPREX®) WITH THE ESTIMATION OF URINARY OESTRIOL EXCRETION DURING PREGNANCY

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Several drugs given to the mother are known to interfere with urinary oestriol or total oestrogen assays during pregnancy Well known in this respect are corticosteroids ampicillin and hexamethylene tetraamine While the two first types of drugs interfere with the production of oestriol hexamethylene tetraamine interferes with the analytical procedure Thus hexamethylene tetraamine during the acid hydrolysis of the urinary conjugates liberates formaldehyde which gives rise to massive destruction of the oestrogens as demonstrated by Touchstone et al (7) and by Sele & Frandsen (6) Recently a new urinary antiseptic containing hexamethylene tetraamine has been introduced in Sweden (Hiprex®) and has gained a widespread use We therefore feel it important to draw the clinicians attention to this problem Without knowledge of the analytical side effect of hexamethylene tetraamine erroneous conclusions might be drawn from falsely low oestriol excretion values

## MATERIAL AND METHODS

Four pregnant women in the last trimester were observed Hexamethylene tetraamine hippurate (Hiprex® Minnesota 3 M Laboratories Ltd Loughborough Leicestersh England) in a dosage of 1 g×2×3 days was given as a routine procedure after urethral catheterization 24 h urinary samples were collected Routine oestriol assays were performed by the method of Frandsen (3) This method includes acid hydrolysis extraction separation of oestriol by solvent partition and Kober colorimetry

Enrichment of urinary steroids prior to acid hydrolysis was carried out by selective adsorption on Amberlite XAD 2 resin as described by Bradlow (1) This procedure includes adsorption of free and conjugated steroids from 7 ml of *urine on a column containing 4 g of Amberlite* XAD 2 washing of the column with water and elution of the steroids with ethanol After evaporation of the ethanol the residue was diluted in 7 ml of water and treated as a urinary sample according to the routine procedure

## RESULTS AND COMMENTS

Treatment with hexamethylene tetraamine caused an 80–90% destruction of the urinary oestriol during *the analyses when they were performed accord*ing to the routine procedure (Table I) Separation of the steroid conjugates from hexamethylene tetraamine by selective adsorption on Amberlite

Table I *Influence of hexamethylene tetraamine therapy on urinary oestriol values during pregnancy*

| Sample | Day relative to treatment with hexamethylene tetraamine | Oestriol excretion mg/24 h Routine procedure | After XAD 2 adsorption |
|---|---|---|---|
| 676-1 | During treatment | 1 0 | 7 0 |
| 676-3 | 2 d after cessation | 7 4 | 9 9 |
| 676 5 | 4 d after cessation | 6 6 | 7 4 |
| 695 1 | 7 d before treatment | 30 1 | – |
| 695 2 | During treatment | 5 6 | 30 7 |
| 695-3 | 2 d after cessation | 59 6 | 38 2 |
| 695 7 | 6 d after cessation | 44 3 | 46 5 |
| 865 1 | 1 d before treatment | 15 9 | 14 9 |
| 865 2 | During treatment | 1 3 | 14.2 |
| 865 3 | During treatment | 2 0 | 13.2 |
| 865-4 | During treatment | ? 3 | 1?.5 |
| 865-5 | 1 d after cessation | 13.5 | 1. 6 |
| 865-6 | 2 d after cessation | 14 7 | 13.3 |
| 1270-1 | During treatment | 2 7 | 15 1 |

XAD 2 resin prior to hydrolysis efficiently eliminates this interference Hexamethylene tetraamine seems to be rapidly eliminated after cessation of the treatment since its destructive effect in the analysis disappears within one or two days

These results once more emphasize the necessity to inform the clinical laboratory about drugs administered to a patient A completely erroneous clinical decision could otherwise be made With appropriate information in this respect correct values might be obtained from contaminated samples with minor methodological modifications In this case such modifications involve a selective adsorption on Amberlite XAD 2 resin (4 5) or the use of enzyme preparations instead of acid for the hydrolysis of the oestriol conjugates It has been shown that hexamethylene tetraamine has no influence upon the results when enzymatic hydrolysis is utilized (2 6 7)

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*Submitted for publication April 11 1975*

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Acta Obstet Gynecol Scand 54 389–391 1975

## LETTER TO THE EDITOR

# RADIATION TREATMENT OF CANCER OF THE CERVIX OF THE UTERUS AT THE RADIUM INSTITUTE IN COPENHAGEN FROM 1951–54

Supplement 30 of this Journal by Rabbe (4) is a most interesting publication and it is noticeable that the methods used for calculation of survival fractions included the Actuarial and the Boag Lognormal Prediction Model methods So often only a Direct method denoted RR by Rabbe is used This was shown in a recent survey of some fifty papers from British and American journals published between 1967 and 1972 which quoted carcinoma cervix survival results (3)

Rabbe refers to Boag s simplified method on page 17 of her article but here the word simplified refers to Boag s discussion of the factor $f_t$ which was not known accurately at the time of the original paper It has subsequently been confirmed (3) as Boag had assumed at the time that $f_t$ can be taken as fixed and equal to about 0 3 with no appreciable error In any case $f_t$ makes very little difference to the estimates obtained Later in the paper how ever the word simplified seems to be used in a different sense to describe the lognormal model with either one or both of the parameters S M fixed and she states (page 19) that *In simplified form Boag s method used on concluded materials gives too great deviations from the results of actu arial method to be acceptable* The evidence on which she bases this statement is that The Actu arial values are generally clearly higher than the Boag values and this is highly significant ($P<$ 0 001) The Rabbe results do in fact show a signifi cant difference between $A$ and $B$ in her Table XII as can be seen from a paired $t$ test For example if the seventeen series consisting of age groups <45 years 45–55 years and >55 years and stages di vided into rad and comb treatments are consid ered then the $t$ statistic equals 3 09 for 16 degrees of freedom This is a significant result at level $P$=0 01

When calculating the $T$ year survival fraction us ing the Actuarial method it is necessary to have observed some of the patients in the series for at least $T$ years The Rabbe series has treatment 1951–1954 with follow up on 1st July 1967 It there fore has maximum and minimum follow up periods of 16½ and 13½ years and the actuarial estimates cannot be extrapolated beyond this point For the later disease stages III and IV where prognosis is poor it is to be expected that there will be very few deaths due to cancer after 13½ years From Table XII of Rabbe (1974) the differences ($A$–$B$) for stage IV comb are 0 0 0 0 and +0 02 and for stage III comb are +0 08 0 04 and +0 01 Using this data a $t$ test shows that the difference between $A$ and $B$ is not significant

For stages I and II where the prognosis is better it is possible that there will be cancer deaths from a recurrence after a period of 13 ½ years has elapsed subsequent to treatment The values of the propor tion cured calculated by Rabbe using the Actuarial method may therefore be expected to come out higher than the estimate of Boag s $C$

A similar study comparing the estimates of the proportion cured by the Actuarial and Boag Lognormal methods for carcinoma cervix has re cently been carried out (3) In this case the results were based on patients treated 1945–1959 with follow up in 1969–1972 with each series being sub divided by stage and five year treatment period 1945–1949 1950–1954 and 1955–1959 The mini mum follow up for the first quinquennium is there fore 20 years compared with the 13½ years of the Rabbe data

The basic data was divided into seven stage I groupings (Total cases in groups 138 101 179 127 265 292 553) nine stage II groupings (Totals 110 68 97 144 143 86 117 152 123) and six stage III

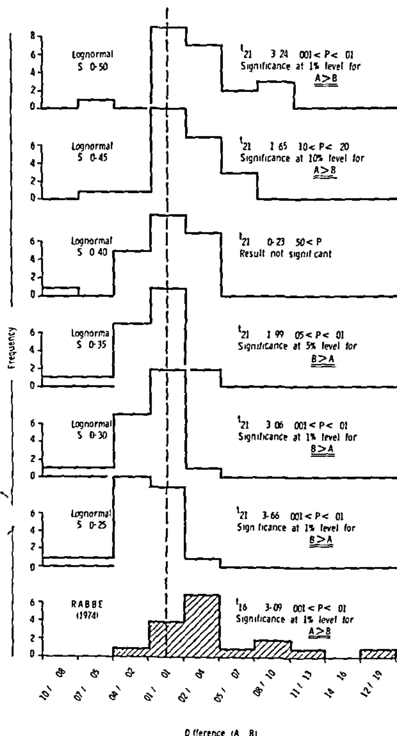



*Fig 1* Frequency distributions of differences (*A*–*B*)

groups (Totals 170 90 114 78 78 76) The Boag Lognormal model was that with a fixed value assumed for the parameter *S* thus leaving only two unknown parameters in the likelihood equation *M* and *C* and a computer program was written which allowed rapid solution of the maximum likelihood equations For each of the twenty two series six Lognormal models were tried with the value of *S* assumed to be 0 25 0 30 0 35 0 40 0 45 and 0 50 It was found that estimates of 10 year and 15 year

survival rates made by actuarial and by Boag Lognormal methods agreed very well and that the 20 year actuarial estimate came close to Boag s $C$ Fig 1 shows at the bottom the spread of $(A-B)$ values in Rabbe s data Above that is shown the spread of $(A-B)$ in the present authors s data worked out for several different values of the fixed parameter $S$ From this diagram is seen that the distribution of differences $(A-B)$ depends upon the value assumed for the Lognormal parameter $S$ It is not correct to assume for *all* series of carcinoma cervix patients that the Actuarial method will always give rise to a higher value for $C$ than the Boag Lognormal method A survey of the present author s data suggests that $S$ lies generally in the range 0 3 to 0 4 for cervix cancer

Boag has previously suggested (2) that the model he set up is perhaps most useful as a framework for extrapolation from incomplete data to 10 15 or 20 year survival figures rather than using $C$ alone as a criterion However on concluded data to use Rabbe s terminology there is in our experience no difference between $C$ and the actuarial estimate of long term survivors

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Acta Obstet Gynecol Scand 54 393–400 1975

# SERUM AND PLACENTAL LACTIC DEHYDROGENASE AND ALKALINE PHOSPHATASE ISOENZYMES IN NORMAL PREGNANCY AND IN PRE ECLAMPSIA

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*Abstract* LDH isoenzymes and heat stable alkaline phosphatase were studied in the serum and placental extract of 70 cases of pre-eclampsia and 10 normal pregnancies as a control The starch gel electrophoretic serum and placental isoenzymogram showed that $LDH_4$ and LDH were the main isoenzymes in the placenta while $LDH_1$ and $LDH_2$ were the main isoenzymes in the serum in pre-eclampsia The electrophoretic serum protein pattern in pre-eclampsia showed increased concentration in the $\gamma$ B and $\alpha$ globulins with decreased albumin fraction while in the placenta the albumin fraction was increased together with a decrease in the $\alpha$ globulins The electrophoretic pattern of serum alkaline phosphatase showed a main band of activity at the B globulin zone in all cases of normal pregnancy and pre-eclampsia In the placenta two additional bands were detected

Human and animal tissue lactate dehydrogenases consist of five principal isoenzymes By electrophoresis they are

(*a*) The fast anodic components $LDH_1$ $LDH_2$ as in the heart kidney and erythrocytes

(*b*) Slow isoenzymes LDH and $LDH_5$ as in the liver skeletal muscle and placenta

(*c*) Isoenzymes of intermediate mobility $LDH_3$ as in the lung adrenal gland and thyroid

The serum lactate dehydrogenase activity is frequently elevated in late pregnancy especially during labour Meade & Rosalki (8) found a relative excess of $LDH_3$ and $LDH_4$ in the maternal serum during labour These are the fractions they had previously found in placental extracts The presence of $LDH_1$ and $LDH_2$ in the extracts is attributed to the red blood cells which cannot be easily separated from this tissue

Hawkins & Whyley (2) stated that $LDH_4$ and $LDH_5$ were the principal isoenzymes in the placenta They also found an increased level of these components in the maternal serum during labour and they suggested that they might be derived from the uterine muscle which was thought to be rich in these fractions

Geyer (1) found that the myometrium of the gravid uterus contained a higher proportion of the slow isoenzymes more than the nonpregnant uterus

Human placental alkaline phosphatase differs from the enzyme of other tissues in being remarkably stable to heat at 70 C for 30 min in the presence of $Mg^{++}$ while alkaline phosphatases from other human tissues are almost completely inactivated when heated at 56 C for the same period (11) This is used as an aid in the identification of the tissue origin of a raised serum alkaline phosphatase (3 13) The elevated serum alkaline phosphatase observed during the third trimester of pregnancy was shown to contain an appreciable heat stable component which was considered of placental origin (6 9 16)

Messer (10) demonstrated that placental dysfunction was associated with drop in the heat stable alkaline phosphatase in serum

Our present investigation is carried to study the level of the different LDH isoenzymes and heat stable alkaline phosphatase in the serum and placental extract in a group of pre-eclamptic patients hoping to localise which fraction of the placental isoenzymes is responsible for the total rise in the serum of toxemic patients

Table I *The description of 20 pre eclampsia and 10 normal pregnancies submitted for isoenzyme es timations both in the placenta and serum*

| Case | No | Mean age in years | Mean parity | Gestosis index |
|---|---|---|---|---|
| Normal preg nancy | 10 | 28 ±3 7 | 2 9±2 6 | – |
| Mild pre eclampsia | 10 | 25 7±5 7 | 1 8±2 6 | 5 0±1 1 |
| Severe pre e lampsia | 10 | 30 5±6 4 | 1 6±2 8 | 7 3±1 2 |

## MATERIAL AND METHODS

Thirty cases of pregnant women were selected from the antenatal ward at Ain Shams University hospitals during the year 1972 and they were classified into three groups as follows (*a*) 10 cases of normal uncomplicated pregnancy *as a control group (b) 10 cases of mild pre-eclampsia and* (*c*) 10 cases of severe pre-eclampsia

In this classification of mild and severe pre eclampsia we followed the standard issued during the Paris Meeting of the Organization Gestosis held in October 1970 Table I describes the three groups of cases

The time of collecting all samples was unified by taking the third stage of labour as the proper time to obtain both maternal blood and the placental samples All cases were delivered vaginally with no difficult interference and anaesthesia if needed was either local infiltration or pu dendal block anaesthesia

*Samples for analysis*

Before delivery maternal blood was withdrawn and kept in a centrifuge tube to clot at room temperature for ½-1 hour then the serum was separated by centrifugation The serum was kept at 4°C until analysis was carried on the same day

The placental wedge was collected after vaginal deliv ery The wedge constituted the whole thickness and it was excised from the centre to the margin of the placenta after draining off as much blood as possible Portions of 5–10 g were washed in ice-cold distilled water blotted weighed and homogenized for 3 minutes with an equal volume of 0 05 M tris HCl buffer pH 7 4 The homogenate was then centrifuged at 3 000 r p m for 20 minutes at 4 C and the supernatant solution was used for analysis

The serum and placental extract were used for assay of the following

1 Total lactic dehydrogenase enzyme (LDH) and the heat stable and heat labile fractions applying the method of Wroblewski & Gregory (17)

2 Heat stable alkaline phosphatase by the method of Kind & King (4)

3 *Starch gel electrophoresis for lactic dehydrogenase* isoenzymes using the horizontal starch gel method of Smithies (14 15) The starch gel was prepared from hydrolysed starch using 13 g/100 ml buffer pH 8 8 The buffer was composed of Tris EDTA and boric acid Each gel was 2×23 cm and 6 mm in thickness Good separation of the isoenzymes was obtained when a potential of 6–8 volts/cm was applied over a period of 14–16 hours with a bridge buffer consisting of boric acid and sodium hydrox ide at pH 8 2

After electrophoresis the starch gel block was sliced with a fine wire along its whole length into two sections

Table II *LDH fractions by both starch gel electrophoresis and heat stability/lability methods in normal pregnancy and pre eclampsia*

H S =heat stable L F =labile fraction Sign =significance I U=international unit N S =not significant

| Serum | Total LDH I U | Heat stable fraction I u | Heat labile fractions I U | LDH isoenzyme % | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | $LDH_1$ | $LDH_2$ | $LDH_3$ | $LDH_4$ | $LDH_5$ |
| *Normal pregnancy* | | | | | | | | |
| Mean | 154 5 | 86 4 | 68 1 | 40 8 | 29 9 | 14 81 | 11 64 | 0 63 |
| S D | ±17 5 | ±5 6 | ±9 6 | ±2 1 | ±2 6 | ±1 5 | ±0 8 | ±0 03 |
| % of the H S to L F | | 55 0% | 44 4% | | | | | |
| *Mild pre eclampsia* | | | | | | | | |
| Mean | 193 3 | 115 98 | 77 32 | 39 11 | 37 18 | 15 2 | 13 5 | 0 314 |
| S D | ±19 9 | ±15 6 | ±10 8 | ±1 1 | ±2 8 | ±0 8 | ±2 2 | ±0 1 |
| Sign of *t* % of H S and L F | *P*<0 001 | 60% | 40% | N S | *P*<0 001 | N S | N S | N S |
| *Severe pre eclampsia* | | | | | | | | |
| Mean | 384 1 | 230 3 | 152 8 | 37 72 | 27 78 | 14 89 | 15 51 | 0 409 |
| S D | ±31 8 | ±17 05 | ±11 05 | ±2 6 | ±0 9 | ±0.5 | ±1 3 | ±0 01 |
| Sign of *t* % | *P*<0 001 | 60% | 39 7% | *P*<0 01 | *P*<0 05 | N S | *P*<0 001 | *P*<0 001 |

Table III *LDH fractions by both starch gel electrophoresis and heat stability/lability methods in normal pregnancy and pre eclampsia*

H S =heat stable L F =labile fraction Sign =significance I U=international unit N S =not significant

| Placenta | Total LDH I U | Heat stable fractions I U | Heat labile fractions I U | LDH isoenzyme % | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | LDH | $LDH_2$ | LDH | LDH | $LDH_5$ |
| *Normal pregnancy* | | | | | | | | |
| Mean | 16? 6 | 97 5 | 65 3 | 13 62 | 17 86 | 15.5 | 28 82 | ?4 2 |
| S D | ±7 68 | ±4 8 | ±3 05 | ±1 0 | ±0 6 | ±0 6 | ±2 1 | ±1 7 |
| % of the H S to L F | | 39 9% | 40 1% | | | | | |
| *Mild pre eclampsia* | | | | | | | | |
| Mean | 181 9 | 110 8 | 69 4 | 1? 23 | 16 36 | 15 81 | 30 82 | 24 98 |
| S D | ±4 68 | ±3 ? | ±1 7 | ±0 4 | ±0 4 | ±0 7 | ±1 4 | ±0 4 |
| Sign of t % of H S and L F | $P<0\ 001$ | 60 9% | 39% | $P<0\ 001$ | $P<0\ 001$ | N S | $P<0\ 02$ | N S |
| *Severe pre-eclampsia* | | | | | | | | |
| Mean | 406 7 | ?84 0 | 12? 7 | 12 57 | 16 41 | 13 43 | 30 2 | 27 39 |
| S D | ±9 03 | ±6 6 | ±2 6 | ±0 4 | ±0 7 | ±1 0 | ±0 6 | ±0 5 |
| Sign of 1% of H S and L F | $P<0\ 001$ | 79 9% | 30 1% | $P<0\ 01$ | $P<0\ 001$ | $P<0\ 001$ | $P<0\ 001$ | $P<0\ 001$ |

each was approximately 3 mm in thickness One of the inner slices was stained for the isoenzymes of either LDH or alkaline phosphatase but the other slice was stained for protein fractions LDH isoenzymes were stained according to the method of Latner & Skillen (7) The alkaline phosphatase isoenzymes were stained as mentioned in the method of Pearse (1?)

A quantitative determination of each isoenzyme was carried by elution of different segments of the starch gel according to the method of Wroblewski (17) for LDH

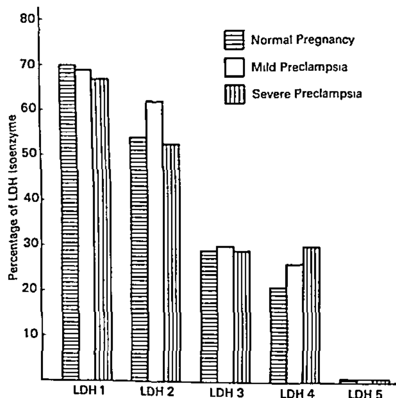



*Fig 1* Serum LDH isoenzyme fractions in normal pregnancy and pre-eclampsia

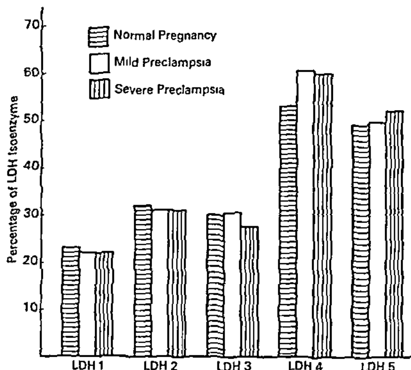



*Fig 2* Placental LDH isoenzyme fractions in normal pregnancy and pre-eclampsia

isoenzymes and Kowlessar et al (5) for the alkaline phosphatase isoenzymes The isoenzymes in the different eluted fractions were assayed colorimetrically according to the previous method for total enzymic activity The peaks of activity can then be demonstrated at different positions along the gel eluted

RESULTS

*Lactic dehydrogenase isoenzymes*

1 *In normal pregnancy* The mean serum total LDH was 154 5±12 2 I U/litre of which 55% are heat stable and the rest are heat labile The mean placental concentration was 162 6±7 7 I U/g of which 59 9% were heat stable and the rest were heat labile

The isoenzyme results indicated that $LDH_1$ and $LDH_2$ were present in the highest concentration in the serum whereas in the placental extract they were at their lowest concentration $LDH_4$ and $LDH_5$ appeared at their highest level in the placenta and at their lowest level in the serum $LDH_3$ level in the serum and in the placenta was nearly the same

2 *In mild pre eclampsia* The mean serum level was 193 3±19 9 I U/litre of which about 61% was heat stable The mean placental concentration was 181 9±4 7 I U/litre of which 60% was heat stable $LDH_1$ and $LDH_2$ showed the highest level in the serum with non significant change from the figures obtained in normal pregnancy Their concentration in the placenta had significantly decreased as compared to the figures of pregnancy The reverse was

Table IV *Heat stable alkaline phosphatase in normal pregnancy mild and severe pre eclampsia*

| Heat stable alkaline phosphatase | Serum Normal pregnancy | Serum *Mild* | Serum *Severe* | Placenta Normal pregnancy | Placenta *Mild* | Placenta *Severe* |
|---|---|---|---|---|---|---|
| Mean±S D | 4 3±_0 | 11 6±1 4 | 17 2±0 9 | 8 5±0 7 | 14 6±0 9 | _2 3±1 9 |
| Test of significance | P<0 001 (Normal vs Mild) | | | P<0 001 (Normal vs Mild) | | |
| | P<0 001 (Normal vs Severe) | | | P<0 001 (Normal vs Severe) | | |

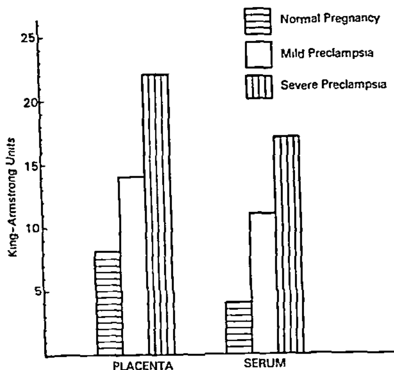



*Fig 3* Heat stable alkaline phosphatase in the serum and placenta of normal pregnancy and pre eclampsia

indicated in $LDH_4$ and $LDH_5$ which showed non significant change from the figures of normal pregnancy in both the serum and the placental extract $LDH_3$ was nearly the same in the serum and placenta.

3 *In severe pre eclampsia* The serum mean value of total LDH was 384 1±31 8 I U/litre of which 60% was heat stable while the placental mean value was 406 6±9 0 I U/litre of which about 70% was heat stable $LDH_1$ and LDH isoenzymes of the serum and placenta showed significant decrease when compared to the normal pregnancy $LDH_4$ and $LDH_5$ had significantly increased in both the serum and the placental extract $LDH_3$ did not show any significant change in the serum but in the placental extract a significant decrease occurred when compared to the corresponding normal pregnancy figures

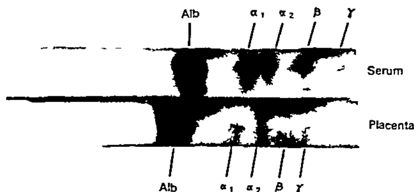



*Fig 4* The electrophoretic pattern of serum and placental proteins in pre-eclampsia

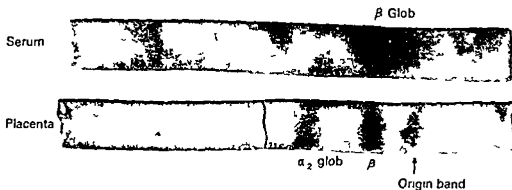



*Fig 5* The electrophoretic pattern of serum and placental alkaline phosphatase in pre-eclampsia

The above results are demonstrated in Tables II and III and also illustrated in Figs 1 and 2

*Heat stable alkaline phosphatase*

1 *In normal pregnancy* The heat stable alkaline phosphatase showed an average of 5 3±1 4 King Armstrong Units (K A U)/100 ml in the serum and 8 5±0 7 K A U/100 mg placenta

2 *In mild pre eclampsia* The mean value of serum level was 11 6±1 41 K A U/100 ml while the placental value was 14 6±0 94 K A U/100 mg and both values of serum and placenta were significantly increased more than the figures of normal pregnancy

3 *In severe pre eclampsia* The mean serum level was 17 2±0 9 K A U/100 ml and the mean placental level was 22 3±1 94 K A U/100 mg Both figures showed a significant increase when compared to normal pregnancy and mild pre eclampsia

The results are summarized in Table IV and illustrated in Fig 3

The electrophoretic serum protein pattern in

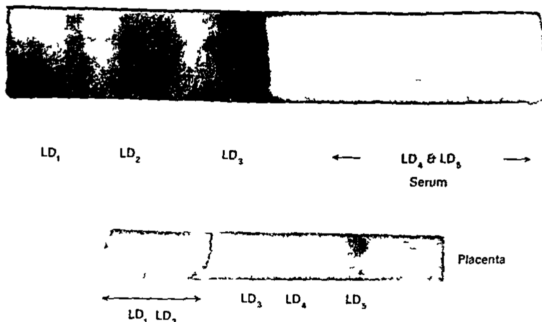



*Fig 6* Starch gel electrophoretic serum and placental LDH isoenzymogram in patients with pre-eclampsia

pre eclampsia showed increased concentration in the γ B and α globulins with decreased albumin fraction while in the placenta the albumin fraction was increased together with a decrease in the α globulins (Fig 4)

The heat stable alkaline phosphatases isoenzymes in the serum appeared electrophoretically as one band at the B globulin region (Fig 5) In case of the placenta three bands appeared of which one band was identical with the serum band while the second band appeared near the origin and the third band in the region of the $\alpha_2$ globulin The starch gel electrophoretic placental LDH isoenzymogram in patients with pre eclampsia is demonstrated in Fig 6

## DISCUSSION

The total lactic dehydrogenase estimation both in the placenta and in the serum of patients suffering from pre eclampsia indicated a highly significant increase in the different grades of toxemia The heat stable fraction of this enzyme was 55% of the total in normal serum and it rose to 60% in the serum and placenta of both grades of toxemia with the exception of the placenta in severe pre eclampsia where it reached 70%

The electrophoretically fast anodic isoenzymes $LDH_1$ and $LDH_2$ decreased in nearly all grades of toxemia in both serum and placenta The change was non significant in the serum of mild pre eclampsia but a significant decrease was noticed in the serum of severe cases On the other hand a highly significant drop was recorded in the figures of the placental extract of all grades of toxemia On considering $LDH_3$ isoenzyme the changes in its concentration in serum and placenta of different grades of toxemia was insignificant except with the placenta of severe toxemia where a highly significant decrease was obtained

The electrophoretically slow moving isoenzymes i e $LDH_4$ and $LDH_5$ which are characteristically synthesized in the placenta and other tissues showed an increase in both the serum and the placental extract of all grades of toxemia in comparison to the normal pregnancy $LDH_4$ showed a non significant increase in the serum of mild pre eclampsia and in the placental extract of the severe group The serum of severe toxemia and the placenta of mild toxemia showed a significant increase of this isoenzyme

$LDH_5$ increased in the serum and placenta in mild pre eclampsia but such rise was non significant In severe cases the increase was highly significant in both the serum and the placental extract

From our data we can conclude that the $LDH_4$ and $LDH_5$ in serum and placenta in different grades of toxemia are the only isoenzymes responsible for the significant increase of total lactic dehydrogenase in both serum and placenta These findings are in agreement with those reported by Hawkins & Whyley (2) The increased level of these isoenzymes may be attributed to increase biosynthesis in the placenta and their release from degenerated placental cells into the blood

As far as the heat stable alkaline phosphatase is concerned the statistical analysis of our results indicated a highly significant increase of the enzyme in both serum and placenta of patients suffering from different grades of pre eclampsia when compared to normal pregnancy Our results prove the previous consideration of many authors (6 9 16) They all stated that the heat stable component of the alkaline phosphatase is of placental origin The heat stable alkaline phosphatase isoenzyme is the main fraction of the total alkaline phosphatase responsible for the significant increase in different grades of toxemia due to placental damage The high level of such isoenzyme in the serum of toxemic patients would mean a serious placental insufficiency

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*Submitted for publication Oct 1 1973*

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Acta Obstet Gynecol Scand 54 401–406 1975

# CONTINUOUS EPIDURAL ANAESTHESIA WITH A LOW FREQUENCY OF INSTRUMENTAL DELIVERIES

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*Abstract* The results of 204 cases of regional analgesia by means of continuous epidural block during labour and delivery are reported The frequency of instrumental intervention using forceps or the vacuum extractor is low less than 15% for primi and multi-gravidas combined although pathological conditions were recognized prior to blockade in almost one third of the parturients Moreover the frequency of emergency Caesarean section has dropped significantly since epidural anaesthesia was introduced into the clinic The 1 min Apgar scores of babies born to mothers given epidural anaesthesia are similar to those of babies born to mothers receiving conventional analgesic agents The amount of blood lost by mothers given epidural anaesthesia was on average 15% less than in a control group It is concluded that the essential feature of our technique of administering continuous regional analgesia is a selective block of small pain fibres with the deep tactile sensations largely intact and the motor pathways virtually unaffected This has been achieved using small doses of bupivacaine 0 25% concentration and meticulous observation of the individual patient

During the last year selective epidural anaesthesia i e blockade of small sensory and autonomic nerve fibres with little or no motor involvement and a restricted segmental spread (4 7) has been administered to more than 200 mothers at Rikshospitalet Oslo We have been able to keep the frequency of instrumental deliveries below 15% primigravidas and multigravidas combined Among the primigravidas 20% were delivered instrumentally the corresponding figure in the group of multigravidas being 7% Caesarean section was performed in 2% of all cases

Thus the frequency of spontaneous deliveries in our series is higher than in any similar account previously published It may be appropriate therefore to report our experience with the method and subject our results to critical appraisal

Continuous epidural block is a potent method for regional analgesia Its lumbar administration effectively relieves pain during labour and delivery (3) This has been amply documented by several authors who have reported almost 100 000 cases of epidural blocks in obstetric practice (3 4 5 8 11 15 18)

Opponents of the method and several proponents as well agree that an increased frequency of instrumental deliveries is inevitable with epidural blocks (1 5 6 9 13 20) This point of view has been challenged by Doughty (7) and Potter & MacDonald (19) who have stated that the method does not increase the frequency of delivery by means of vacuum extraction or forceps In fact the relative number of forceps deliveries has been reported to diminish despite a marked increase in the number of epidural blocks provided (7)

## MATERIAL AND METHODS

The present series of 204 patients was collected from September 1972 to September 1973 during which period the total number of deliveries was 1971 Among our patients 133 were primigravidas the remaining 71 patients being multigravidas In the following account these two groups have been treated separately None of our patients had received epidural anaesthesia on any previous occasion Further information on our series is shown in Table I

Relief of pain during labour is the principal purpose of epidural anaesthesia in obstetrics However in many instances candidates for epidural anaesthesia have to be selected on the basis of additional indications because a large number of pathological pregnancies are admitted to this university clinic

The additional indications include

1 Slow progress with or without abnormal uterine activity
2 Pre-eclampsia
3 Prematurity and/or intra-uterine distress

Table I *Characteristics of parturients studied*

| Parturients | Number of cases | Spontaneous labour | Induced labour* |
|---|---|---|---|
| Primiparae | 133 | 110 | 23 |
| Multiparae | 71 | 51 | 20 |
| Total | 204 | 161 | 43 |

Induced by means of oxytocin primiparae 20 multiparae 16 Induced by means of prostaglandin $-F_2\alpha$ primiparae 3 multiparae 4

4 Maternal diseases or disabilities i e cardiac incompensation polyarthritis epilepsy and other diseases of the central nervous system in which hyperventilation was judged undesirable
5 Severe fetal malformations and fetal death *in utero*
6 Analgesia requested in order to perform forceps delivery or vacuum extraction

The mothers belonging to groups 5 and 6 11 altogether have not been considered in this study since an instrumental mode of delivery had been decided upon prior to the epidural blockade The distribution of various pathological conditions represented among 63 of the mothers (30 5%) is listed in Table II Except for one case of breech delivery and one of twins all mothers had vertex deliveries Epidural anaesthesia was administered with the patient lying in the right or the left lateral position The loss-of resistance technique was employed exclusively using a Tuohy needle with a syringe containing 0 9% saline Puncture was performed at the $L_3$-$L_4$ level (101 cases) or at the $L_4$-$L_5$ level (103 cases) A test dose of 5 ml 0 25% bupivacaine without adrenaline (Marcain® Bofors) was sed during the first 10 months Later this procedure was anged and 5 ml of 1% lidocaine (Xylocain® Astra) was d as a test dose due to its more rapid onset of action and horter duration

In all mothers an intravenous infusion was started before anaesthesia Ringer solution was given routinely except in cases of pre-eclampsia or cardiac disease Such patients received 5 5% glucose i v The blood pressure was measured before administration of anaesthesia and every fifth minute during the first half hour Later it was checked at 10-15 min intervals Twenty nine patients were continuously monitored using a Hewlett Packard Cardiotocograph with Hon s spiral scalp electrode and a transcervical catheter connected to a pressure transducer

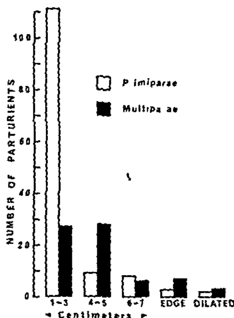



*Fig 1* Relationship between cervical dilatation and start of epidural blockade in primi and multiparae

## RESULTS

Epidural anaesthesia was administered when the mother was in established labour with painful contractions The corresponding degree of cervical dilatation is illustrated in Fig 1 It appears that the group of primigravidas requested pain relief at an earlier stage in labour than those who previously had given birth However the great majority of the latter group complained of pain and received their epidurals before the cervix had dilated beyond 5 cm

The time elapsing from blockade to delivery the total amount of bupivacaine given (test dose included) and the number of doses injected are shown in Table III On average the duration of epidural block in our primigravidas lasted for approximately 1 hour 45 min longer than among the multigravidas Also the total amount of bupivacaine given and the

Table II *Pathological conditions present prior to epidural blockade*[a]

| Parturients | Pre eclampsia | Uterine inertia | Intra uterine distress | Pre maturity | Maternal di ability |
|---|---|---|---|---|---|
| Primiparae | 6 | 20 | 13 | 2 | 3 |
| Multiparae | 2 | 8 | 6 | 2 | 7 |
| Total | 8 | 28 | 19 | 4 | 10 |

[a] The total number of patients exceeds 63 because certain patients had more than one diagnosis

Table III *Anaesthesiological details*

| Parturients | Parameter | Interval (hrs) blockade–delivery | Total amount of Marcain (mg) | Number of doses | mg Marcain/dose | Doses/hr | mg Marcain/hr |
|---|---|---|---|---|---|---|---|
| Primiparae | Range | 0 75–16 50 | 17–315 | 1–13 | | | |
| Multiparae | | 0 25– 6 25 | 25–198 | 1– 6 | | | |
| Primiparae | Mean | 3 94 | 98 0 | 3 5 | 25 2 | 0 9 | 22 6 |
| Multiparae | | 2 26 | 70 6 | 2 6 | 26 7 | 1 7 | 31 2 |
| Primiparae | Standard deviation | 2 56 | 60 6 | 2 0 | | | |
| Multiparae | | 1 42 | 33 2 | 1 1 | | | |

number of doses administered were higher among the primigravidas These findings are probably due to the longer period of time of blockade When the total amount of bupivacaine given is compared with the duration of the epidural anaesthesia the primigravidas received a smaller amount of the local anaesthetic agent than the group of multigravidas did per unit of time This observation shows that a satisfactory level of analgesia may be achieved just as easily among primigravidas as is the case with multigravidas Thus the difference between primigravidas and multigravidas is probably related to an earlier onset of pain among the former Moreover when analgesia with an adequate segmental spread is satisfactorily obtained at an early stage the need for large top up doses seems correspondingly reduced

The upper level of analgesia was determined in every case by the pin prick method The segmental spread is illustrated in Fig 2 It shows an even distribution centring around the Th8 dermatome

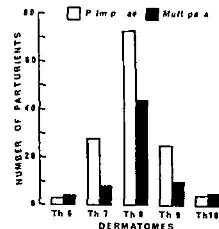



*Fig ?* Distribution of segmental spread of analgesia its upper level determined by the pin prick method

with very few cases extending beyond the Th7 segmental level and none above Th6 the results being very similar in the two groups of patients

The circulation remained remarkably stable throughout the period of epidural blockade In fact only one patient developed hypotension defined as a systolic blood pressure drop below 100 mmHg from normotensive values necessitating treatment by means of a pressor agent (ephedrine sulphate) The incident resulted in a synchronous episode of fetal bradycardia reported elsewhere (10)

The modes of delivery are shown in Table IV More than 83% of the total number of deliveries were spontaneous less than 15% were delivered by means of vacuum extraction or forceps Among the primigravidas 20% were delivered instrumentally the corresponding number in the multigravidas group was less than 6% Two per cent of all cases had a Caesarean section

The blood lost at delivery and during the first 2 hours *post partum* is collected routinely by the attending midwives Admittedly this is a crude but clinically useful estimate of the amount of blood lost A comparison between patients given epidural anaesthesia and a similarly sized group receiving conventional analgesics in a period during which regional block analgesia was not available shows a mean blood loss of 198 ml in the former against 231

Table IV *Modes of delivery*

| Parturients | Spontaneous deliveries | Instrumental deliveries | Caecarean section |
|---|---|---|---|
| Primiparae | 104 (78%) | 26 (20%) | 3 (2%) |
| Multiparae | 66 (93%) | 4 (6%) | 1 (1%) |
| Total | 170 (84%) | 30 (15%) | 4 (2%) |

Percentages estimated to nearest full figure

Table V *Anaesthetist s evaluation of analgesic effect*

| Stage of labour | Sensory effects | Primi parae | Multi parae | Total |
|---|---|---|---|---|
| I Established labour | Complete sensory blockade | 9 | 6 | 15 |
| | Complete pain relief | 117 | 59 | 176[a] |
| | Incomplete pain relief | 4 | 4 | 8 |
| | Asymmetrical pain relief | 1 | 1 | 2 |
| | Persistent back pain | 2 | 0 | 2 |
| | No effect | 0 | 1[b] | 1 |
| II Expulsatory phase | No bearing-down sensation | 25 | 9 | 34 |
| | Contractions recognized | 108 | 62 | 170 |
| | Perineal analgesia at expulsion | 124 | 68 | 192 |
| | Perineal pain at expulsion | 9 | 3 | 12 |

[a] Nine patients referred to this group had one or several unblocked segments initially but with subsequent analgesia
[b] Abrupt delivery upon epidural injection

ml in the latter These findings agree well with the observations of Moir & Wallace (12)

The analgesic effects obtained are shown in Table V during established labour and in the expulsatory phase respectively It appears that pain was completely relieved in 93 % of the cases In this figure we have included the patients who in addition to pain relief experienced a full sensory blockade Contractions were recognized during expulsion by 83 % of the parturients while approximately 6 % reported pain Among those who did not feel contractions at all (17 %) there was as expected an increased frequency of instrumental deliveries

The 1 min Apgar score was determined by the midwives in routine cases When complications were encountered it was determined by the resident anaesthetist paediatrician or obstetrician The results are presented in Table VI A comparison of the mean 1 min Apgar scores between the patients given epidural anaesthesia and a control group receiving conventional analgesic agents turned out to be exactly the same 9 23 in both groups

In addition to the one episode of acute hypotension already described there was one incident of

Table VI *Apgar score after one minute*

| Apgar score | Primiparae | Multiparae | Total |
|---|---|---|---|
| 10–9 | 115 | 62 | 177 |
| 8–7 | 13 | 3 | 16 |
| 6–5 | 3 | 3 | 6 |
| 4–3 | 1 | 2 | 3 |
| 2–0 | 1 | 1 | 2 |

dural tap This is the same rate (0 5 %) as experienced previously in surgical patients (17) The patient received abundant amounts of fluid and was allowed to stay out of bed on the second day She did not complain of headache

Bladder disturbance was observed after delivery in 2 patients Their difficulties vanished spontaneously within a couple of days however Six patients reported low backache which may be attributable to their epidural injections Venous bleeding into the Tuohy needle was observed in some cases Injections were not given unless the bleeding ceased Moreover we have seen no evidence of venous cannulation

The patient s response to pain relief by means of epidural blockade was evaluated on the first or second day after delivery (Table VII) Approximately 86 % of our patients reported full satisfaction whereas an additional 13 % felt that they had been helped The discrepancy between the 93 % whose epidurals were classified as giving complete pain relief (Table V) and the 86 % who expressed full satisfaction may be due to pain experienced between top up doses The one patient who reported that she did not benefit from her epidural anaesthesia delivered abruptly upon injection of the local anaesthetic agent into the epidural space (Table V) One patient in whom complete relief of pain was achieved complained that she missed the feeling of having given birth which she had experienced on two previous occasions In order to obtain general information with respect to the acceptance of epidural anaesthesia the patients were invited to express their desire for the same type of analgesia on any

Table VII *Patients assessment of the efficiency of epidural block reported on the first or second day after delivery*

| Assessment | Primiparae | Multiparae | Total |
|---|---|---|---|
| Fully satisfied | 114 | 61 | 175 |
| Helped | 19 | 8 | 27 |
| No benefit | 0 | 1 | 1 |
| Satisfied but deprived | 0 | 1 | 1 |

later occasion Two patients (1%) would decline a subsequent offer of epidural blockade

## DISCUSSION

Epidural anaesthesia although eminently effective in relieving pain during labour and delivery is not a routine procedure in most clinics Two factors are largely responsible for the cautious attitude encountered in many Scandinavian hospitals Firstly introduction of continuous lumbar blockade as a general service depends on a staff with adequate training in its obstetric management Secondly an apprehension rooted in the belief that epidural anaesthesia invariably causes the frequency of instrumental deliveries to increase prevails among obstetricians (9) Moreover some authors have expressed the opinion that continuous lumbar anaesthesia imposes a serious threat to the fetus (21)

The results presented here do not support these discouraging predictions Thus an instrumental delivery had to be resorted to in 20% of the primigravida cases the corresponding figure among the multigravidas being less than 6% (Table IV) Nevertheless if one plainly compares the frequency of instrumental deliveries before epidural anaesthesia was introduced into this hospital with our results a definite increase appears to have taken place in both groups Our patients however represent a selected group with a high frequency of pathological features before blockade (Table II)

Therefore a high proportion of instrumental deliveries was to be anticipated among these patients To decide whether epidural anaesthesia does in fact promote an increased frequency of instrumental deliveries our data should be matched with the corresponding figures in a similar period of time during which continuous epidural anaesthesia was not available Such a comparison has been made (Table VIII) the 12 months prior to its introduction serving as a control against the one year period discussed in this paper The number of births and the frequency of instrumental deliveries appear remarkably constant Thus the introduction of continuous epidural anaesthesia did not change the ratio between the two modes of delivery This indicates that a disproportionately large number of patients who would be needing an instrumental delivery in any event received a lumbar blockade and thus appear in our series This would account for the over representation of deliveries by means of vacuum extraction or forceps here the frequency of instrumental deliveries being correspondingly reduced among the rest of the patients in the period studied Moreover it should be noted that we do not allow the second stage in labour to exceed 1 hour

We have found no evidence to support the notion that epidural anaesthesia causes a high incidence of fetal malrotation subsequent to undue relaxation of the muscles in the pelvic floor (20) This we believe may be attributed to our use of the selective mode of providing regional analgesia Fetal welfare is further confirmed in a generally high Apgar score after 1 min in spite of the many pathological features of the patients selected for regional block This result supports the findings of Noble et al (16) who reported a higher 5 min Apgar score among babies born to mothers with epidural anaesthesia as compared with a similar group given conventional analgesia Furthermore the patients themselves appear to have benefitted additionally from the epidural blockade their average blood loss being 15% lower than that of the control group This agrees well with observations previously published (12)

Only 4 of the mothers with epidural anaesthesia had to undergo Caesarean section (Table IV) three on an indication of slow progress and one due to

Table VIII *Equal periods of time before and after introduction of epidural anaesthesia compared*

| Classifications of parturients | Total number of deliveries | Number of instrumental deliveries | Per cent instrumental deliveries |
|---|---|---|---|
| 1 9 1971–31 8 197 | | | |
| Primiparae | 881 | 67 | 7 6 |
| Multiparae | 1 119 | 15 | 1 3 |
| 1 9 197[illegible]–31 8 1973 | | | |
| Primiparae | 916 | 71 | 7 7 |
| Multiparae | 1 005 | 13 | 1 3 |

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| 6–5 | 3 | 3 | 6 |
| 4–3 | 1 | 2 | 3 |
| 2–0 | 1 | 1 | 2 |

Acta Obstet Gynecol Scand 54 407–410 1975

# PRESENCE OF ANTISPERM ANTIBODIES IN FERTILE AND INFERTILE PERSONS

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*Abstract* The sera of 70 infertile and 30 fertile couples were tested for the presence of sperm agglutinins with Kibrick s macroagglutination technique and Boyden s haemagglutination test 15 7% of the couples studied showed the presence of sperm agglutinins (by Kibrick s method) of which 5 7% were from males and 10 0% were from females By the haemagglutination test 13 % of the couples studied were found to possess sperm agglutinins of which 3% were from males and 10% were from females 30 fertile men and women studied for sperm agglutinins were found to be negative by both methods It was also observed that these two tests detected different types of sperm agglutinins The cervical mucus samples from 45 females (15 fertile and 30 infertile) were tested for sperm agglutinins with a mucus penetration test 23 1 16 5 and 57 4% of the samples from infertile females showed 0-degree 1-degree and 2-degree penetration respectively In case of samples from fertile females 6 6 13 2 and 79 2% showed 0-degree 1-degree and 2-degree penetration respectively Addition of serum from infertile females to cervical mucus from the infertile female increased the 0-degree penetration percent cases to 50% as compared with 23 1 when only cervical mucus was used Addition of serum from fertile females or horse serum or pure albumin or globulins did not increase the 0-degree penetration cases

It has been shown statistically that 7–10% of couples are infertile (1 8 11) The causes of infertility may be the male factor tubal factor or ovulation failure Some of the cases of infertility have not been related to any of these factors In some such cases the presence of antisperm antibodies have has demonstrated (9 13 15 18) The presence of circulating sperm antibodies has been demonstrated (3) in the serum of females showing no organic causes of infertility Fjallbrant & Obrant (6 7 8) showed the presence of antisperm antibodies in 37% of sterile males Sperm agglutinating antibodies have also been demonstrated in cervical secretions (16 19)

In the present investigation the incidence of sperm agglutinins in the serum of sterile males and females and the cervical mucus of females has been studied The effect of serum on the sperm penetration of cervical mucus has also been studied

## MATERIALS AND METHODS

Four groups of cases were investigated in order to study the incidence of antisperm antibodies in the sera and cervical mucus of sterile and fertile persons

*Group I* included 70 infertile couples
*Group II* included sera of 15 normal males and 15 normal females
*Group III* included the cervical mucus of 30 infertile females
*Group IV* included 15 normal females whose cervical mucus was tested

### *Collection of blood cervical mucus and sperms*

(*a*) Collection of blood 5 ml blood from veins was drawn under aseptic conditions and the serum was separated

(*b*) Collection of cervical mucus Procedures of Parish et al (1967) and Iacobelli et al (1971) were followed

(*c*) Collection of semen Semen obtained from healthy fertile males was collected The count and motility of sperms were noted after liquefaction and the samples with counts more than 20 million/ml and motility 70% were considered to be normal

### *Detection of sperm agglutinating antibodies in serum and cervical mucus*

For the detection and titration of sperm agglutinating antibodies the methods of Boyden (2) Stavitsky (17) and the macroagglutination test of Kibrick et al (12) were followed

The method of Fjallbrant (6 7 8) was followed for the detection of sperm agglutinating antibodies in cervical mucus

Table I. *Titre of natural antihuman sperm antibodies in blood of fertile and infertile couples detected by Kibrick's test and Boyden's haemagglutination technique*

| Types of cases | No. of cases with positive reaction and their titre 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | Total | % | No. of negative cases | Total cases |
|---|---|---|---|---|---|---|---|---|---|
| *Kibrick's test* | | | | | | | | | |
| Infertile males | 4 | – | – | – | – | 4 | 5.7 | 66 | 70 |
| Infertile females | 1 | 2 | 4 | – | – | 7 | 10.0 | 63 | 70 |
| Fertile males | – | – | – | – | – | – | 0 | 15 | 15 |
| Fertile females | – | – | – | – | – | – | 0 | 15 | 15 |
| *Boyden's haemagglutination technique* | | | | | | | | | |
| Infertile males | | | 1 | 1 | – | 2 | 3 | 68 | 70 |
| Infertile females | | | | 5 | 2 | 7 | 10 | 63 | 70 |
| Fertile males | – | – | – | – | – | – | 0 | 15 | 15 |
| Fertile females | – | – | – | – | – | – | 0 | 15 | 15 |

## RESULTS

Presence of natural antisperm antibodies in sera of both fertile and infertile couples was studied by using Kibrick's and Boyden's techniques. The results (Table I) showed that according to Kibrick's method natural antisperm antibodies were present in 5.7 and 10.0% of the infertile males and females sera respectively. The highest titre of antibodies was found to be 1:4 and 1:16 in males and females respectively. The results were compared with the data obtained by Boyden's method (Table I). It was observed that 3% and 10% of the sterile males and females respectively showed positive results. The highest antibody titre was found to be 1:32 and 1:64 in males and females respectively.

When sterile males and females showing a positive reaction by Kibrick's and Boyden's methods were compared, it was observed (Table II) that 4 males and 7 females showing a positive reaction by Kibrick's method gave negative results by Boyden's method and vice versa.

Table II. *Patient showing antisperm antibodies by Kibrick and Boyden technique*

| S. No. of male patient | Positive cases by Kibrick's | Boyden's |
|---|---|---|
| 1 | + (1:4) | – |
| 2 | + (1:4) | – |
| 3 | – | + (1:16) |
| 4 | + (1:4) | – |
| 5 | – | + (1:16) |
| 6 | + (1:4) | – |
| Female patients | | |
| 1 | + (1:16) | – |
| 2 | – | + (1:32) |
| 3 | – | + (1:64) |
| 4 | – | + (1:32) |
| 5 | + (1:4) | – |
| 6 | + (1:16) | – |
| 7 | – | + (1:32) |
| 8 | + (1:8) | – |
| 9 | + (1:8) | – |
| 10 | + (1:16) | – |
| 11 | – | + (1:64) |
| 12 | + (1:16) | – |
| 13 | – | + (1:32) |
| 14 | – | + (1:32) |

Presence of sperm agglutinating antibodies in the cervical mucus of both fertile and infertile females was studied and the results (Table III) showed that 23.1, 16.5 and 57.4% of the infertile cases studied showed 0 degree, 1 degree and 2 degree penetration of the cervical mucus respectively, whereas in case of cervical mucus from fertile females 6.6, 13.2 and 79.2% of the cases gave 0-degree, 1 degree and 2-degree penetration respectively.

The effect of different types of sera on the sperm penetration of cervical mucus was studied. The results are given in Table IV. It was observed that upon addition of sera from a sterile female to the cervical mucus of a sterile female (in equal volumes) 0-degree penetration was found in 50% of the cases as compared with 23.1% of the cases observed when only cervical mucus was used. Similarly 1-degree penetration was found in 30% cases compared with 16.5% cases in presence of serum. Addition of serum from a fertile female or male, horse serum albumin, bovine serum or globulins from a normal person did not show any effect on the penetration of sperms in the cervical mucus of infertile females.

Table III *Detection of antisperm antibodies in cervical mucus by Fjallbrant method*

| Penetration of cervical mucus from | Degree of penetration 0 | 1 | 2 | Total |
|---|---|---|---|---|
| Infertile females | 7 | 5 | 18 | 30 |
| Fertile females | 1 | 2 | 12 | 15 |

## DISCUSSION

Immunological factors have been shown to explain infertility in some otherwise unexplained cases due to the presence of antisperm antibodies in the seminal fluid (Weil et al 1956 Edward 1963) in cervical mucus (Solish et al 1961 Fjallbrant 1965) and in the blood of both sterile males and females (Rumke & Hellinga 1959 Fjallbrant 1968) Results discussed in Table I confirm the earlier observations (Ansbacher et al 1967) that antisperm antibodies are present in a small percentage of sterile cases but these results differ from those reported by Franklin & Dukes (1964) and Schwimmer et al (1967) Moreover these methods detect two different types of sperm agglutinins (Table II)

The presence of antisperm antibodies in the cervical mucus has been shown (Silson 1954 1956 Rumke & Hellinga 1959 Solish et al 1961 Straus 1961) but the number of sterile cases showing the presence of these antibodies is small and this has also been shown in the present results (Table III) With the addition of serum from a sterile female to the cervical mucus of sterile females 50% of them

Table IV *Detection of antisperm antibodies in cervical mucus in presence of serum*

| Penetration of cervical mucus of infertile female in presence of | Degree of penetration 0 | 1 | 2 | Total |
|---|---|---|---|---|
| blood serum (1 1) | | | | |
| from infertile females | 15 | 9 | 6 | 30 |
| serum from horse | 4 | 10 | 11 | 25 |
| serum from fertile | | | | |
| females | 1 | 1 | 13 | 15 |
| albumins | 2 | 1 | 12 | 15 |
| globulin | 1 | 3 | 11 | 15 |

*Scoring system*

0 degree = All sperms seen at periphery

1 degree = Most of the sperms at periphery and few in centre

2 degrees = Sperms equally distributed to centre and periphery

showed 0 degree penetration of sperms These results show that with the addition of serum of a sterile female the detection of antibodies inhibiting sperm penetration can be detected in more cases It is also possible that the presence of serum enhances the function of antisperm antibodies in cervical mucus The factor in the serum which may be responsible for this enhanced reaction has to be studied But on the basis of the present study it is suggested that serum may be added to the cervical mucus before determining the penetration of sperms

## ACKNOWLEDGEMENTS

The authors wish to thank Dr A N Gupta Associate Professor Post graduate Institute of Medical Education and Research Chandigarh Dr R D Bansal Assistant Professor Snowdon Hospital Simla and Dr (Mrs) Raj Lakshmi Lecturer Lady Reading Hospital Simla for helping in obtaining cervical mucus samples blood and semen samples

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*Submitted for publication April 22 1974*

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Acta Obstet Gynecol Scand 54 411–415 1975

# DIFFERENT METHODS OF RECONSTRUCTION AFTER VULVECTOMIES FOR CANCER OF THE VULVA

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*Abstract* Direct wound closure with or without flap-plasty after vulvectomy gives satisfactory cosmetic and functional results Split thickness skin-grafts seem to be of limited value in the repair of the defects

Cancer of the vulva may develop at any location in the vulvar region Occasionally the origin may be multifocal with extension to adjacent organs especially the urethra vagina and anus In 6 6% of the cases (2) it coexists with other types of cancer

A detailed description of the vulvar carcinoma with symptoms histology staged classification treatment and prognosis is given in papers by Edsmyr Green et al Held & Engeler and Kottmeier (2 3 4 6)

The methods of treatment of the primary tumour vary In 1930 Stoeckel (8) summarized methods of treatment used up till then

Different forms of radiation therapy often in combination with surgical procedures are still used at many places

The Radiumhemmet method was introduced in 1922 by Berven It consists of electrocoagulation of vulvar tissue followed by secondary healing Occasionally it gave astonishingly good cosmetic and functional results However it often made coitus impossible because of stenosis of the vaginal orifice There is a risk of symphysial damage and recto-vaginal fistula formation (2) Some weeks after the electrocoagulation radiation therapy over the groins was given

Radical vulvectomy though an old method is being used more frequently It gives good cosmetic and functional results especially if followed by a primary reconstruction

Twombly (9 10) described his en bloc dissection technique for superficial deep inguinal and femoral nodes followed by radical vulvectomy Twombly closed the vulvar defect by direct suturing but stressed the need for plastic procedures later in some cases

In Sweden Johanson & Lewin (5) reported satisfactory cosmetic and functional results using split thickness skin graft for the repair of the vulvar defect

An interesting nursing care study given by Pears (7) described the problems associated with secondary healing following vulvectomy

This report does not include the conclusions concerning survival time or frequency of recurrence because our material was not uniform and the observation times were too short This is a report covering different techniques used for the repair of the vulvar defect after a radical vulvectomy and the results obtained from a functional and cosmetic point of view by utilizing different principles of reconstruction

## MATERIAL AND METHODS

At the Karolinska Hospital in Stockholm Sweden between 1967–1972 patients with squamous cell carcinoma of the vulva have been operated upon jointly by gynecological and plastic surgeons and followed-up by the Department of Gynecology at the Radiumhemmet Only histologically confirmed squamous cell carcinomas are included in this study In some patients the extent of invasion of this squamous cell carcinoma was limited and local and in some cases more extensive growth was noted

Since most of our patients are referred from other hospitals this study includes patients with various forms of treatment before referred to us Some had been treated by coagulation some by small limited excisions A few pa



*Fig 1* Age distribution

tients had local radiation therapy to the inguinal areas and a few who were too old for radical surgery had been treated by local surgery and postoperative irradiation

Our main treatment has been radical vulvectomy followed by removal of the superficial inguinal nodes bilaterally En bloc dissection of both inguinal areas is performed which includes the skin the subcutaneous tissue and the inguinal nodes A wide vulvar excision is carried out including both the inguinal areas even if the primary lesion is small and unilateral The inguinal area dissection can be performed later

The series consists of 78 patients with the age distribution shown in Fig 1 Many of our patients were poor risks surgically One of the patients included in this study had myocardial infarction and died three weeks postoperatively Epidural anesthesia was used in almost all cases

The vulvectomies are performed by the gynecological surgeon Starting from a point high up over the pubic bone a broad oval incision is made encircling the vulva and including at least 3 cm of normal tissue lateral to the lesion The incisions extend and in some cases surround the anus depending on the site of the lesions Separate incisions are made around urethra and vagina The vaginal incisions are usually performed about 15 mm above the orifice or higher if the orifice has been invaded by cancer Thereafter the vulval tissue is dissected free from the periosteum of the pubic bone and from the fascia of the perineal musculature The inner anal sphincter is left undisturbed in all cases Meticulous haemostasis is obtained utilizing diathermy free hand ligatures and stitching with catgut especially around the remnants of the crura clitoridis and in the cavernous region The vaginal wall is mobilized 2 cm proximally The reconstruction of the defect is then done by the plastic surgeon and the following methods are used

*Method 1* The skin and subcutaneous fat surrounding the incision is mobilized A direct suture of the raw edges is performed with non absorbable suture material (Fig 2)

Technically it is important to start the suturing in the pubic region using big mattress sutures and stretching the skin backwards thus facilitating the repair around urethra vagina and anus where the defect is generally largest and the closure problems are more pronounced The closure is facilitated further if the thighs are adducted during the suturing In most cases suction drains are used bilaterally for 2–4 days postoperatively An indwelling catheter is left in the bladder for approximately one week

*Method 2* The excision of the lesion is performed in the same way as in method 1 The defects are so large that a direct suture is inappropriate especially in the posterior part (Fig 3)

The anterior part of the defect can be closed by direct suture but the posterior defect needs two triangular flaps one of them based anteriorly and the other one based posteriorly Suction drains and catheter are used as in method 1

*Method 3* For the closure of major defects double

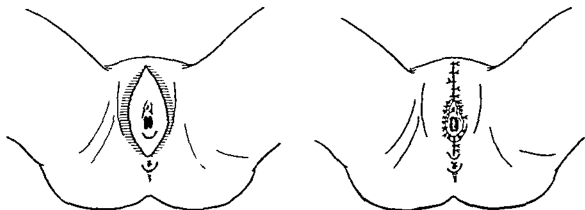
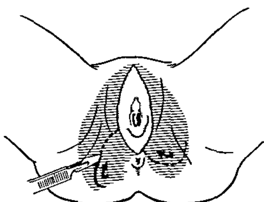


*Fig 2* Reconstructive method no 1 Direct suture after mobilization



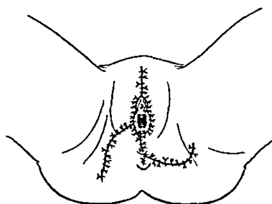
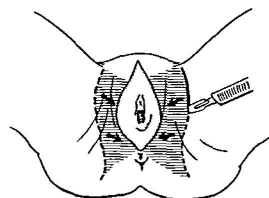


*Fig 3* Method 2 The defect is closed by using two local flaps



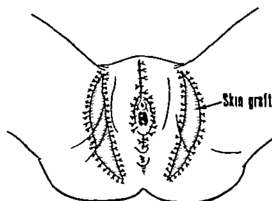
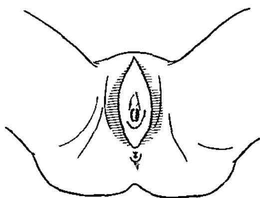



*Fig 4* Method 3 For closure of major defects double based flaps from the inner sides of the thighs are used The secondary defects are covered with split thickness skin grafts



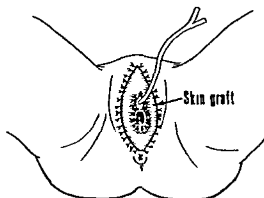



*Fig 5* Method 4 Defect covered with split thickness skin-grafts

Table I *Results in 78 cases of cancer of the vulva*

| Reconstruction | Bad | Acceptable | Very good | Excellent |
|---|---|---|---|---|
| 1 Direct suture (44 cases) | 0 | 1 (2%) | 9 (20%) | 34 (78%) |
| 2 Single based flaps (13 cases) | 0 | 0 | 4 (30%) | 9 (70%) |
| 3 Double based flaps (7 cases) | 0 | 0 | 2 (29%) | 5 (70%) |
| 4 Split skin grafts only (14 cases) | 8 (57%) | 6 (43%) | 0 | 0 |

based flaps from the lateral parts of the perineum and the inner side of the thighs are used The secondary defects on the inner sides of the thighs are closed by split thickness skin grafting (Fig 4)

However often by undermining the thigh skin and sliding it anteriorly it is possible to make direct sutures of the flaps which have been stretched backwards Small dog ears anteriorly on the thighs are removed later when the groin dissection is performed When this method for closure is used the groin dissection should be delayed for 2–3 weeks Such a dissection may impair the circulation in these flaps

*Method 4* The defect after the excision is covered with split thickness skin grafts If the haemostasis is perfect the graft may well be sutured in place during the operation and a tie-on pressure dressing could be applied (Fig 5)

If the haemostasis is doubtful delayed grafting the following day is preferred A urinary catheter is used for 8–14 days while the graft is healing Partial loss of the graft is not uncommon in these cases

The first method described above is used mainly for defects of moderate size The second method is used for defects of medium size and the third method is used for very large defects Method 4 may be used in all cases Method number 1 takes the least amount of time for operation and method number 4 takes the most

*Inguinal area* Both inguinal areas are operated on in nearly all cases By oval excision a suitable amount of skin and subcutaneous tissue *is excised down to the fascia* A meticulous undermining of the skin gives possibility for excision of all subcutaneous fat from the original oval excision From this incision the dissection in the vulva region to the lateral part of the groin can be performed and the inguinal glands including the gland of Rosenmuller can be excised easily Deep inguinal lymph nodes are not disturbed The inguinal area exploration can be performed at the same time as the vulvectomy if the reconstruction is performed according to the methods 1 2 or 4 depending upon the general state of the patient The inguinal area dissection should be postponed for two weeks when method 3 is used since there is a definite risk of disturbing the circulation to the flaps *However* many of these patients are old and it is wise to postpone the inguinal area dissection for some weeks after the vulvectomy until healing has taken place in the vulvar region The patient should be ambulated before the groin dissection to reduce the risk of thrombosis

## RESULTS

As is shown in Table I method 1 was used in 44 cases method 2 in 13 cases and methods 3 and 4 in 7 and 14 cases respectively

The results judged from a cosmetic and functional stand point have been graded on a four point scale from bad to excellent

The results with direct suturing including flap-plasties were classified as very good or excellent in 98% of 64 patients The 14 cases reconstructed only with split thickness skin grafts had their end results classified as bad in 57% or acceptable in 43% Another 25 cases have been operated upon using methods 1 2 and 3 with satisfying results since this study was finished

## DISCUSSION

In this series split thickness grafts contracted with the soft deeper layers of the perineum Initially delayed healing was often noted in these cases creating a tender skin grafted pubic bone and contractures around the orifices of vagina and urethra *thus hindering coitus Sometimes* further operative procedures were needed to achieve satisfactory sexual activities and urination

Direct suturing including flap plasties resulted in rectal vaginal and urethral orificies of acceptable width and function Flap plasty treated sexually active patients were able to have intercourse even if the sensitivity of the area was lowered

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*Submitted for publication May 14 1974*

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Acta Obstet Gynecol Scand 54 417–423 1975

# STUDIES IN CHOLESTASIS OF PREGNANCY

## V *Gallbladder Disease Liver Function Tests Serum Lipids and Fatty Acid Composition of Serum Lecithin in the Non pregnant State*

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*Abstract* Women from an earlier series of cholestasis of pregnancy (CP) were called for a retrospective study to consider presence of gallstones in the gallbladder (evaluated by cholecystography) liver function tests serum lipids and lipoproteins and the relative fatty acid composition of serum lecithin (as determined by GLC) The attendance in the retrospective study was 60% Estimated on the total series (assuming that none of the non responders would have a positive X ray) the incidence of gallbladder disease was 23 7% Among women with previous cholestasis of pregnancy and with a positive X ray finding no characteristic changes in liver function tests or serum lipids were revealed The women with positive X ray had however a lower α lipoprotein cholesterol than women with negative X ray A characteristic finding among women with positive X ray was furthermore the low relative content of palmitic acid (16 0) in serum lecithin Also women with negative X ray had a lower relative content of palmitic acid than controls It is suggested that the low palmitic acid content is expressive of an influence on liver lecithin synthesis pathways and that the serum lecithin fatty acid composition reflects similar changes in bile lecithin The basic defect in CP influencing liver lecithin synthesis might be the primary cause for disturbed cholesterol solubility in bile and of the frequent occurrence of gallstones in CP

During pregnancy intrahepatic cholestasis chole stasis of pregnancy (CP) is seen in predisposed women The etiology of CP is still unknown but estrogens have been proposed as a contributing factor by provoking an insufficiency in the excretory ability of the liver

Earlier studies have indicated a high incidence of cholecystopathia in CP (12 17 25) Even normal pregnancy has been considered cholestatic (24 27) and a female preponderance of gallbladder has lead to the suggestion that female gonadal steroidal hormones interfere with bile formation leading to lithogenic bile The phosphoglyceride lecithin participates in the dissolving of cholesterol in bile Also the quality of bile lecithin might be of importance to the solubility of cholesterol in bile (19 23 26)

In cholestasis during pregnancy but also in women with a past history of CP the fatty acid composition of serum lecithin has a characteristic pattern (13 21) It is assumed that these characteristic changes in serum lecithin fatty acid composition reflect metabolic influences on lecithin synthesis in the liver

The aim of the present study was to elucidate the prevalence of cholecystopathia (by means of cholecystography) in the non pregnant state in women with previous CP and also to investigate the liver function tests serum lipids and the fatty acid composition of serum lecithin in relation to gallbladder disease

## MATERIALS AND METHODS

*Clinical series (Fig 1a)* The earlier series (14) of cholestasis of pregnancy (CP) comprised 41 women Three women had left the community at the time of this study The remaining 38 women were called in for a retrospective study in the non-pregnant state 8–71 months after delivery During the preceeding pregnancy seventeen women had been designated as pruritus gravidarum (PG) and twenty one as hepatosis of pregnancy (HP) (14) In the retrospective studies these groups were called previous PG and previous HP'

Twenty three women (mean age 27 8, range 21–35 years) responded to the calls eleven with previous PG and twelve with previous HP (Fig 1b and c) All women had been free of pruritus since the preceeding delivery

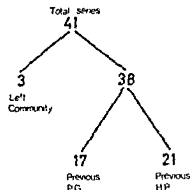



*Fig 1a* Patient series

Two women had a past history of cholecystectomy and were excluded from the X ray examination (*cholecystography*) Two women were using oral contraceptives and one woman was (at the time of the study) 14 days past her expected menstruation She later proved not to be pregnant These latter three women were excluded from the gas liquid chromatography (GLC) part of the study which then consisted of nine women with previous PG and eleven women with previous HP

Eighteen healthy non pregnant women (mean age 26 2 range 19–34 years) not using oral contraceptives served as a control series Blood samples were drawn on the first or the second day of the menstrual cycle

*Cholecystography* On the evening before the examination the women were given Teletrast® orally (after eating a fat meal) and the examination was carried out on the following morning

*Blood sampling* Blood samples were drawn from an ante-cubital vein in the fasting state in the morning After clotting the blood was centrifuged at 2 500×g for ten min and serum was recovered immediately frozen and at −20°C in glass tubes with teflon screw caps

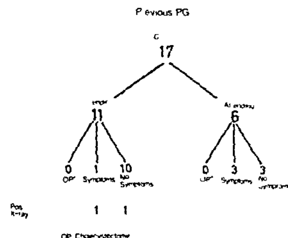



*Fig 1b* Gallbladder disease in the subgroup of previous PG

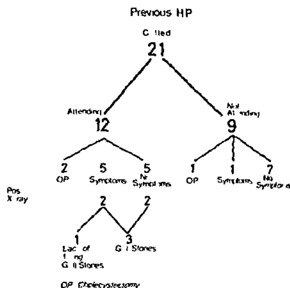



*Fig 1c* Gallbladder disease in the subgroup of previous HP

*Liver function tests* Serum total bilirubin (normal <1.2 mg/100 ml) alkaline phosphatase (normal <260 U/l or <8 Buch Units) SGOT (ASAT) (normal <17 U/l) SGPT (ALAT) (normal <17 U/l) and Simplastin A (normal 70–130%) were performed at the Laboratory of Clinical Chemistry according to standard methods

*Serum lipids* Serum triglycerides were determined according to Carlson (7) total cholesterol by the method of Cramer & Isaksson (8) The cholesterol content (15) of high-density lipoproteins (HDL) was quantified after the precipitation of very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) with manganese chloride and heparin (5)

*Gas liquid chromatography (GLC) procedure* Preparation of lipid extracts separation of lipids by thin layer chromatography on Silica gel and isolation of lecithin and phosphoglyceride spots and preparation of fatty acid methyl esters were performed as described by Olegård & Svennerholm (20)

GLC of methyl esters and conversion from weight to mole per cent were performed as described in an earlier publication (13)

*Quantification of serum lecithin* Serum lecithin was quantified from the fatty acid content (obtained by GLC) using a nomogram The equation $y=1.66x+75$ where $y$ is the serum lecithin content as determined by lipid phosphorus (3) and $x$ the amount of lecithin calculated from fatty acid analyses satisfied the experimentally determined relationship (13)

The absolute amount of lecithin (in mg/100 ml) with each particular fatty acid was obtained from fatty acid (weight per cent)×lecithin (mg/100 ml) The absolute amount of lecithin is important to consider when comparing the relative fatty acid composition in states with different serum lecithin levels (22)

*Statistical methods* Conventional methods were used for the calculation of means standard deviations and

Table I *Liver function tests serum lipids and lipoproteins in pruritus gravidarum (PG n=11) and hepatosis of pregnancy (HP n=12) and in the same women 8–21 months after delivery (previous PG n=11 and previous HP n=12)*

Mean ±S E M Values for liver function tests and Simplastin A from control at the Laboratory of Clinical Chemistry Values for lipids from control series of normal non pregnancy women ($n$=18) Alk Phos =alkaline phosphatase Simpl =Simplastin A Chol =total cholesterol TG=triglycerides $\alpha$ LP-chol =$\alpha$ lipoprotein-cholesterol

| | | Bilirubin (mg/100 ml) | Alk phos (Buch units U/l) | GOT (U/l) | GPT (U/l) | Simpl (%) | Chol (mg/100 ml) | TG (mg/100 ml) | $\alpha$ LP chol (mg/100 ml) | Lecithin (mg/100 ml) |
|---|---|---|---|---|---|---|---|---|---|---|
| I PG (pregnant) $n$=11 | $\bar{X}$ | 0.44 | 10 | 19 | 15 | 139 | 268 | 216 | 72 | 213 |
| | S E M | 0.1 | 1.3 | 3.4 | 4.1 | 12 | 13 | 30 | 6 | 11.2 |
| II HP (pregnant) $n$=12 | $\bar{X}$ | 1.3 | 16 | 61 | 90 | 170 | 311 | 259 | 58 | 241 |
| | S E M | 0.23 | 1.00 | 6.9 | 15.3 | 10 | 24 | 15 | 5 | 20.6 |
| III Previous PG $n$=11 | $\bar{X}$ | 0.9 | 14? | 10 | 7 | 95 | 199 | 74 | 68 | 197 |
| | S E M | 0.1 | 16 | 2.0 | 2.54 | 3.6 | 10 | 13 | 5 | 10.5 |
| IV Previous HP $n$=12 | $\bar{X}$ | 0.8 | 160 | 14 | 12 | 82 | 224 | 66 | 66 | 186 |
| | S E M | 0.1 | 21 | 3.0 | 3.9 | 5.1 | 9 | 10 | 5 | 6 |
| Control series | | <1.2 | <8<br><160 | <17 | <17 | 70–130 | 206 | 54 | 60 | 198 |
| I vs III | $P$< | 0.001 | – | 0.05 | N S | 0.01 | 0.001 | 0.001 | N S | N S |
| II vs IV | $P$< | 0.05 | – | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | N S | 0.05 |
| III vs IV | | N S | N S | N S | N S | N S | N S | N S | N S | N S |

standard error of means Student s t test was used to study differences between groups Values of $p \leq 0.05$ were considered statistically significant (4)

## RESULTS

1 *Symptoms of gallbladder disease (Figs 1b and 1c)* In the total series (cf Fig 1a) of women with previous HP ($n$=21) three women had a past history of cholecystectomy and five additional women complained during pregnancy of gallbladder distress altogether eight women (38%)

In the total series of previous PG ($n$=17) four women (24%) had complained of gallbladder distress

2 *Cholecystectomy and positive X ray (Figs 1b and 1c)* In the total series of women with previous CP ($n$=38) three women (8%) had a past history of cholecystectomy Si women (16%) had positive findings on X ray of he gallbladder (five revealed gallstones and one lack of filling simultaneously with presence of gall tones)

In the retrospective series subgroup of previous HP ($n$ 12) (cf Fig 1a) six women (50%) revealed p X ray ($n$=4) or a past history of cholecystectomy ($n$=2) and in previous PG ($n$=11) (cf Fig 1a) two women revealed positive X ray

3 *Liver function tests (Tables I and II)* In the retrospective series subgroups of previous HP and previous PG in the non pregnant state the mean values for serum bilirubin alkaline phosphatase SGOT and SGPT and Simplastin A were within normal limits As compared to corresponding values during pregnancy significant differences were found particularly in HP ($n$=12) in serum bilirubin ($p<0.05$) SGOT ($p<0.001$) SGPT ($p<0.001$) and *Simplastin A ($p<0.001$) (Table I)*

In the non pregnant state liver function tests in previous PG ($n$=11) and previous HP ($n$=12) did not differ (Table I)

In previous CP ($n$=23) in the non pregnant state no significant differences in liver function tests were found in women with positive X ray as compared to women with negative X ray (Rable II)

In the same group of women with previous CP ($n$=23) during pregnancy liver function tests did not differ among women with subsequent positive and negative X ray

4 *Serum lipids and $\alpha$-lipoprotein ($\alpha$-LP) cholesterol (Tables I and II)* In previous HP and previous CP in the non pregnant state serum

Table II *Liver function tests serum lipids and lipoproteins in women with previous CP with positive (n=6) and negative (n=15) X ray of the gallbladder*

Mean ±S E M Individual data from two women with previous CP earlier cholecystectomized are included Alk phos =alkaline phosphatase Simpl =Simplastin A chol=total cholesterol TG=triglycerides α LP-chol =α lipoprotein cholesterol and Lec=lecithin

| PG+HP | | Bili rubin (mg/ 100 ml) | Alk phos (U/l) | GOT (U/l) | GPT (U/l) | Simpl (%) | Chol (mg/ 100 ml) | TG (mg/ 100 ml) | α LP chol (mg/ 100 ml) | Lec (mg/ 100 ml) |
|---|---|---|---|---|---|---|---|---|---|---|
| I Gall Pos $n=6$ | $\bar{X}$ | 0 90 | 170 | 12 | 13 | 91 | 209 | 66 | 52 | 18[illegible] |
| | S E M | 0 2 | 38 | 4 5 | 7 2 | 6 | 13 | 16 | 5 | 10 |
| II Gall neg $n=15$ | $\bar{X}$ | 0 83 | 138 | 15 | 8 | 82 | 215 | 67 | 72 | 198 |
| | S E M | 1 | 13 | 5 7 | 2 0 | 3 | 10 | 10 | 4 | 9 |
| Cholecystectomized $n=2$ | | 0 6 | 197 | 9 | 4 | 68 | 2[illegible]9 | 86 | 70 | 166 |
| | | 0 3 | 200 | 36 | 26 | 84 | 220 | 120 | 75 | 185 |
| I vs II | | N S | N S | N S | N S | N S | N S | N S | N S | N S |

cholesterol triglycerides and α LP cholesterol were not different from those values in control series Serum lipids in previous PG and previous HP did not differ (Table I)

In previous CP ($n=26$) in the non pregnant state serum lipids were not different in women with positive and negative X ray α LP cholesterol was lower ($p<0$ 01) in women with positive X ray (Table II)

In the same group of women with previous CP during pregnancy serum lipids and α LP cholesterol did not differ among women with positive and negative X ray

5 *Relative fatty acid composition of serum lecithin* In previous HP ($n=12$) in the non pregnant state sum of fatty acids of linoleic acid series ($n=6$) was lower ($p<0$ 05) than in previous PG ($n=11$) 35 8 and 38 0 mole% respectively In previous PG (197 mg/100 ml) as compared to previous HP (186 mg/100 ml) no significant difference in absolute amounts of serum lecithin was found

In *previous CP* ($n=20$) in the non pregnant state as compared to during pregnancy as described earlier (Ref ) significant differences in 16 0 (palmitic acid) 18 0 (stearic acid) 18 1 (oleic acid) 18 2 (linoleic acid) and 20 4 (arachidonic acid) occurred

Table III *Relative composition of major fatty acids of serum lecithin in women with previous CP with positive (n=6) and negative (n=12) X ray of the gallbladder and in control series of normal non pregnant women*

Mean ±S E M Individual data from two women with previous CP earlier cholecystectomized and one women with previous CP the X ray positive group with lack of filling of gallbladder are included

| Previous CP | | 16 0 | 16 1 | 18 0 | 18 1 | 18 2 | 20 3 | 20 4 | 22 6 | 18–22 n-6 | Lecithin (mg/100 ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I X ray negative $n=12$ | | 28 5 | 0 75 | 14 5 | 11 7 | 26 5 | 2 5 | 7 7 | 4 7 | 37 2 | 198 |
| | | 0 41 | 0 06 | 0 28 | 0 38 | 0 65 | 0 30 | 0 28 | 0 44 | 0 56 | 8 7 |
| II X ray positive $n=6$ | | 27 7 | 0 83 | 14 9 | 11 8 | 26 8 | 2 4 | 7 2 | 5 3 | 36 6 | 18[illegible] |
| | | 0 33 | 0 07 | 0 59 | 0 60 | 1 56 | 0 16 | 0 40 | 0 74 | 1 17 | 10 3 |
| III Lack of filling $n=1$ | | 28 7 | 0 77 | 14 8 | 11 6 | 22 6 | 2 3 | 7 7 | 7 8 | 42 7 | 165 |
| IV Cholecystectomized $n=2$ | | 30 4 | 0 86 | 13 8 | 10 9 | 22 3 | 2 3 | 10 3 | 5 7 | 35 5 | 166 |
| | | 27 7 | 0 85 | 14 8 | 13 4 | 27 0 | 2 4 | 6 1 | 4 8 | 35 6 | 185 |
| V Normal non pregnant $n=18$ | | 29 7 | 0 74 | 13 9 | 11 7 | 28 5 | 2 1 | 6 9 | 4 2 | 36 2 | 198 |
| | | 0 30 | 0 05 | 0 23 | 0 19 | 0 69 | 0 15 | 0 31 | 0 26 | 0 4[illegible] | 4 0 |
| I vs II | $P<$ | 0 10 | N S | N S | N S | N S | N S | N S | N S | N S | N S |
| I vs V | $P<$ | 0 05 | N S | N S | N S | N S | N S | N S | N S | N S | N S |
| II vs V | $P<$ | 0 01 | N S | 0 10 | N S | N S | N S | N S | N S | N S | N S |

Table IV *Relative composition of major fatty acids of serum lecithin during pregnancy in women with CP with subsequent positive (n=2) and negative (n=6) X ray of the gallbladder*

Mean±S E M Individual data from one women earlier cholecystectomized are included

| | 16 0 | 18 0 | 18 1 | 18 2 | 20 4 | 22 6 | 18–22 n-6 | Lecithin (mg/100 ml) |
|---|---|---|---|---|---|---|---|---|
| X ray negative n=6 | 35 1±1 02 | 9 9±0 59 | 15 4±0 96 | 21 2±0 9 | 6 7±0 50 | 5 1±0 30 | 31 6±0 93 | 288±51 3 |
| X ray positive n=2 | 32 6<br>33 1 | 8 5<br>11 0 | 17 3<br>13 1 | 23 8<br>23 8 | 6 0<br>7 6 | 5 2<br>4 1 | 32 8<br>34 9 | 324<br>235 |
| Cholecystectomized n=1 | 35 1 | 11 3 | 10 9 | 30 2 | 4 8 | 2 5 | 38 3 | 254 |

In previous CP ($n=20$) in the non pregnant state women with positive X ray ($n=6$) showed a tendency for lower ($p<0\ 10$) 16 0 (palmitic acid) than women with negative X ray ($n=12$) (Table III)

In previous CP in the non pregnant state women with positive X ray ($n=6$) showed a lower content ($p<0\ 01$) of 16 0 (palmitic acid) than control series ($n=18$) Women with negative X ray ($n=15$) were also lower ($p<0\ 05$) in 16 0 (palmitic acid) than controls In two women with a past history of cholecystectomy and in one woman with a lack of filling at X ray 16 0 (palmitic acid) content was similar to that of the women with negative X ray (Table III)

*In previous CP* ($n=9$) *during pregnancy* women with positive X ray ($n=2$) showed a tendency for a lower content of 16 0 (palmitic acid) than women with negative X ray ($n=6$) and than one woman with a past history of cholecystectomy (Table IV)

## DISCUSSION

Women from an earlier series of cholestasis of pregnancy (CP) have in the present publication been studied in the non pregnant state (8–21 months after delivery) During pregnancy CP had been divided into a subgroup with more advanced liver damage hepatosis of pregnancy (HP) and a milder form (with less influence on liver function tests) pruritus gravidarum (PG) Eight out of 21 women (38%) with HP and four out of 17 women (24%) with PG had during pregnancy complained of gall bladder distress i e upper abdominal pain provoked by a fatty meal

Of those 38 women with previous CP called in for the retrospective study 23 responded and 21 had a gallbladder X ray cholecystography performed Two of those women who responded to the call and one non responder had a past history of cholecystectomy Six out of twelve (50%) of the women with previous HP had either a positive X ray or a past history of cholecystectomy Two out of 11 women with previous PG had a positive X ray Since only four of the non responders ($n=15$) had complained of gallbladder distress during pregnancy it might be suspected that those women who were not attending the study had less gallstones than those women who responded to the gall Thus if it was assumed that all non responders would have a negative X ray the prevalence of gallstones in the total series ($n=38$) was nine women—a minimum of 23 7%—with verified gallbladder disease This prevalence of gallbladder disease in CP is in agreement with data by others (17 12 25) In a population sample of women age 19–29 years a total of 19% revealed gallstones at autopsy (16)

During pregnancy HP was characterized by abnormal liver function tests (cf 14) In the non pregnant state women with previous HP (as well as women with previous PG) showed on average normal function tests Although none of the women attending the retrospective study had experienced pruritus after delivery two women in the subgroup of previous HP ($n=12$) and one woman with previous PG ($n=11$) had elevated (>17 U/l) SGOT and/or SGPT There was however no difference in liver function tests between women with positive and negative X ray neither in the non pregnant state nor during pregnancy

Women with previous CP and positive X ray revealed in the non pregnant state lower $\alpha$ LP cholesterol ($p<0\ 01$) than those with negative

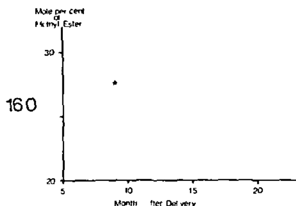



*Fig 2* Palmitic acid relative content of serum lecithin in women with previous PG ($n=9$) and previous HP ($n=11$) in relation to time after termination of a cholestatic pregnancy =previous PG ●=previous HP

X ray When compared to controls women with previous CP and positive X ray had a lower ($p<0.01$) serum lecithin palmitic acid (16 0) concentration However also women with negative X ray (and a past history of CP) showed lower ($p<0.05$) serum lecithin palmitic acid than controls Two women with a past history of cholecystectomy and one woman with lack of filling at the X ray had palmitic acid concentrations in serum lecithin comparable to those in women with negative X ray

If it can be assumed that serum lecithin relative *fatty acid composition is an expression of either* influences on liver lecithin synthesis or availability of specific fatty acids and furthermore that the ı lecithin fatty acid composition reflects that of ın in liver and bile (1) certain considerations in relation to the present finding can be made The low serum lecithin palmitic acid concentration in women with CP and positive gallbladder X ray might either be a secondary phenomenon to the preceding pregnancy the cholestasis or the gallbladder disease or be a primary event Pregnancy is characterized by a relatively high concentration of palmitic acid in serum lecithin (22) The difference in palmitic acid concentration in women with positive and negative X ray findings was however not due to sampling at different times after delivery (Fig 2)

It was then tempting to link the low serum lecithin palmitic acid concentration to the presence of gallbladder disease The synthesis of lecithin in the liver by the Kennedy pathway (pathway I) gives rise to lecithins with palmitic acid (16 0) in 1 position and linoleic acid (18 2) or oleic acid (18 1) in 2 position (2 19) *In vitro* experiments with the addition of bile acids to liver slices (6) as well as *in vivo* studies in man (19) indicate that bile acids enhance liver lecithin synthesis via pathway I A reduced return of bile acids to the liver e g by an interrupted enterohepatic circulation (EHC) would possibly lead to a depression of this synthesis pathway The present data in women with gallbladder disease of a low serum lecithin palmitic acid concentration give support for this explanation Furthermore also CP *women with a negative X ray* showed a characteristically lower serum lecithin palmitic acid concentration than controls This might indicate that in addition to an interrupted EHC in the presence of gallbladder disease women prone to CP might have a basic defect causing a characteristically low palmitic acid concentration in serum lecithin

Generally speaking in the bile the solubility of cholesterol is dependent on quantitative changes in bile acids and lecithin (10) Moreover a growing body of evidence indicates that qualitative changes in bile lecithin i e in lecithin fatty acid composition would interfere with the solubility of cholesterol (19 23 26) It is therefore tempting to suggest that a primary defect in liver lecithin synthesis possibly due to hormonal influences might be the cause of cholesterol precipitation in the bile and a frequent cause of gallstones in women with CP

## ACKNOWLEDGEMENTS

Mrs Margareta Medberg is acknowledged for technical assistance and Miss Annika Höfde for typing the manuscript

This project was supported by grants from the Swedish Medical Research Council (B74-19X 2100-19A) and the Swedish Oleo Margarine Association for Nutritional Research

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*Submitted for publication May 15 1974*

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Acta Obstet Gynecol Scand 54 425–436 1975

# PHOSPHOLIPIDS AND CREATININE IN AMNIOTIC FLUID IN RELATION TO GESTATIONAL AGE

## I Normal Pregnancy

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*Abstract* 185 samples of amniotic fluid were obtained from 174 healthy women with uncomplicated pregnancies in the 15th–43rd week of pregnancy by abdominal amniocentesis or by puncture of the amniotic sac via an amnioscope The concentrations of creatinine (183 cases) phospholipid phosphorus and the ratio of lecithin/sphingomyelin (L/S ratio 130 cases) were determined The treatment of the amniotic fluid samples (centrifugation filtration and lipid extraction) and the methods for the determination of L/S ratio were examined and the accuracy and limitations of the latter method were defined and discussed The relative amounts of lecithin and sphingomyelin as well as the L/S ratio and creatinine concentration were closely correlated to gestational age A L/S ratio of ≥2 25 indicated all women to be ≥34th week of pregnancy 64% of them had reached the 38th week A creatinine concentration of ≥1 8 mg% corresponded to ×≥35th week of pregnancy while 75% of the women had reached at least the 38th week With a L/S ratio ≥2 25 and a creatinine concentration ≥1 8 mg% of all women were ≥ the 36th week 71% of the women ≥ the 38th week fulfilled these criteria A combination of the two parameters seems to be of a great value in discrimination between gestational ages < the 36th week and ≥ the 38th week

Gestational age is usually estimated from the first day of the last menstrual period Not infrequently however women have irregular periods which may obscure the picture Bleeding may also occur during the first months of pregnancy In all these cases conventional criteria for determining the gestational age are very uncertain and the usual formula cannot be applied

This necessitates the use of other criteria for the determination of gestational age Some information can be gained from the estimation of uterine size during pregnancy and the time of first fetal movements The gestational age can also be estimated by X ray examination (11 34 37) but this method is not very accurate above all if the fetus is small for dates near term (36 51) The same applies to ultrasonic examination (9) Cytological examination of amniotic fluid cells stained with Nile blue sulphate has also been used to determine gestational age (7 8 31) but its value has been questioned (18 38) Determination of creatinine concentration in amniotic fluid is considered to be of greater practical value (46)

In the last few years there has been a great deal of interest in the lipid concentration and composition of amniotic fluid It has long been known that the alveoli of the lung are coated with a surface tension lowering material of which dipalmitoyl lecithin is an important constituent (12 13 41) From the lung this material diffuses out into the amniotic fluid (5) In addition to lecithin amniotic fluid also contains smaller amounts of sphingomyelin and some other phospholipids (3) The origin of the latter is however uncertain It was recently shown by Gluck et al (29) that the ratio of the concentration of lecithin to sphingomyelin (L/S-ratio) could be used to estimate the maturity of the human fetal lung

In this investigation we have chosen to study three parameters for the determination of gestational age in uncomplicated pregnancy the lecithin sphingomyelin ratio (L/S ratio) phospholipid phosphorus and the creatinine concentration and have related them to the known gestational age

## MATERIAL

Originally 206 samples of amniotic fluid were obtained from 195 women between the 15th and the 43rd week of gestation After having discarded all samples containing blood or meconium 185 samples from 174 women remained The gestational age was counted from the first day of the last menstrual bleeding (thus in the 35th week means *more than* 34 but not 35 completed weeks) It was considered

Table I *Phospholipid concentration and composition in centrifuged and filtered amniotic fluid*

| Centrifugal force (g min) | Amniotic fluid 40 weeks Phospholipid concentration (µg lipid P/ml) | Phospholipid* composition (%) PC | Sph | Other | Amniotic fluid 18 weeks Phospholipid concentration (µg lipid/P ml) | Phospholipid composition (%) PC | Sph | Other |
|---|---|---|---|---|---|---|---|---|
| 1 500 | 8 1 | 76 2 | 5 5 | 18 3 | 1 07 | 28 8 | 41 6 | 29 7 |
| 20 000 | 4 5 | 76 3 | 3 9 | 19 8 | 0 76 | 32 5 | 39 1 | 30 1 |
| 20 000+filtration | 1 9 | 74 1 | 4 4 | 21 5 | 0 34 | 30 0 | 42 7 | 27.3 |

PC=phosphatidyl choline Sph=sphingomyelin Other represents phospholipids with greater $R_f$ s than phosphatidyl choline

certain when the following criteria were fulfilled (1) regular menstruation for at least the last 4 months preceding the pregnancy (2) interval between menstrual periods of 25–30 days (3) normal length of last menstrual bleeding (4) gynecological examination before the 20th week of gestation at which time the size of the uterus should correspond to the estimated gestational age (5) fetal movements at expected time and (6) an expected rate of growth of the uterus during the pregnancy The samples were obtained from all women fulfilling the above criteria with uncomplicated pregnancies (i e not suffering from toxemia hypertension diabetes or isoimmunization etc ) who entered the hospital in labour from January 1972 until May 1973 Also included were women admitted for therapeutic abortion or with transient premature labour and also women examined for possible immunization of pregnancy where immunization later could be excluded

## METHODS

Amniotic fluid was obtained by abdominal amniocentesis as described by Wiklund (60) or by puncture of the amniotic sac under visual control through the amnioscope Usually about 20 ml of amniotic fluid were obtained It was centrifuged for 10 minutes at 2 000 *g* in an International model CS centrifuge and then filtered through filter paper (Munktell no 005 Grycksbo pappersbruk AB Sweden) In most cases this produced a clear fluid A portion of the samples was frozen and kept at −18°C until analysed for phospholipid content and L/S ratio According to Ekelund et al (19) the samples can be stored in this way with no change in phospholipid concentration and composition Creatinine concentration was determined in an autoanalyzer in another portion of the sample within a day of its collection according to Jaffe s method (10) In two cases creatinine determination failed for technical reasons and in 55 cases the determination of L/S-ratio was not done because of insufficient amounts of liquor or in a few cases for technical reasons

Centrifugation of the amniotic fluid samples is carried out routinely by all investigators Cells and cell debris are removed in this way but unfortunately so is a considerable fraction of the phospholipids (Table I) Filtration of the amniotic fluid also removes a considerable amount of the phospholipids in our case around 50% (Table I) This is less than was reported by Nelson (43) but may be due to properties of the filter paper and/or to whether filtration was performed before or after centrifugation since both procedures may remove the same type of particles

Lipids from 2–5 ml portions of the filtered amniotic fluid were extracted according to a modification (3) of the procedure of Bligh & Dyer (6) The chloroform phases were taken to dryness under nitrogen and dissolved in 1 ml chloroform Acetone precipitation (29) was omitted since it does not precipitate the lecithin and the sphingomyelin quantitatively Lipid phosphorus (44) was determined in aliquots of the extracts as described by Ames & Dubin (1) with the modification that the digestion was carried out using concentrated perchloric acid on a sandbath at 170–200°C for 20 min Thin layer chromatography was performed on 20×20 cm glass plates covered with a 0 25 mm thick layer of silica gel H (AB Merck Germany) according to Skipski et al (54) Aliquots of the lipid extracts containing 0 6–1 0 µg lipid phosphorus were applied to the thin layer plates as spots with a diameter of 0 4–0 6 cm Standard mixtures containing known amounts of rat liver lecithin and ox brain sphingomyelin (total lipid phosphorus concentration 10–20 µg/ml) were applied in similar amounts (about 1 µg/spot) The spots were visualized by spraying the plates with 48% (w/v) $H_2SO_4$ in $H_2O$ and charring at 170–190°C A typical result is shown in Fig 1 In this solvent system the $R_f$ s of lecithin and sphingomyelin are low (Fig 1) When we tried to increase the $R_f$ s by increasing the water content of the solvent system (not shown) sphingomyelin separated into two spots This is known to occur with other solvent systems as well (56) and should be considered as a possible cause of discrepancies in L/S-ratio measurements between different laboratories

The height and width of the lecithin and sphingomyelin spots were measured with a sliding caliper (which gives a precision down to 0 1 mm) and their product was taken as a measure of the amount of substance in the spot A ratio of the amount of lecithin over the amount of sphingomyelin (L/S ratio) was then calculated (29 55) In some experiments the purpose of which was to determine phospholipid composition or fatty acid composition of lecithin the plates were sprayed with 2% (w/v) iodine in methanol or 0.2% (w/v) dichlorofluorescein in 95% ethanol to visualize the

lipids The spots corresponding to the different phospholipids were scraped off and the phospholipids eluted by the method of Arvidsson (2) Lipid phosphorus was then determined as described above Methyl esters of lecithin fatty acids were prepared by transmethylation overnight in 2 % (v/v) $H_2SO_4$ in absolute methanol at 65°C The methyl esters were purified by silicic acid chromatography prior to gas chromatography (20) Gas chromatographic analysis of the methyl esters was performed as previously described (21)

Meconium was obtained from a fetus during delivery (breech presentation) To determine dry weight a portion of the material was dried to constant weight in an oven at 100°C The rest was homogenized in 20 volumes of chloroform/methanol 2 1 (v/v) A chloroform extract of the lipids was then prepared according to Folch et al (23) Cholesterol (59) and cholesterol ester (59) lipid phosphorus (1) fatty acid (15) and ester bonds (57) were then determined Thin layer chromatography on the phospholipids was carried out as described above

A fraction rich in dipalmitoyl lecithin was prepared from adult human lung samples taken at autopsy originally for other purposes by the method described by Frosolono et al (25) The lipids were extracted according to Folch et al (23) Thin layer chromatography and gas chromatography were carried out as described above

As the amniotic fluid samples were obtained either by abdominal amniocentesis (124 cases) or by puncture of the amniotic sac through an amnioscope (61 cases) it was possible that the method used might in some way influence the results This was tested in 13 cases where amniotic fluid was obtained with varying methods on two separate occasions the time lapse being 2–72 h Statistical analysis showed no significant difference ($p > 0.05$) between the two methods with regard to L/S-ratio total phospholipid concentration and creatinine concentration

*The method of determining L/S ratio used in this report* has the advantage that no expensive and complicated

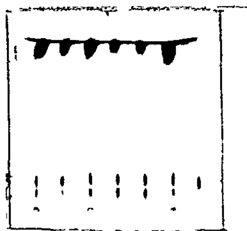


*Fig 1* Thin layer chromatogram of amniotic fluid phospholipids The two lower spots are lecithin (average $R_f = 0.19$) and sphingomyelin (average $R_f = 0.11$)

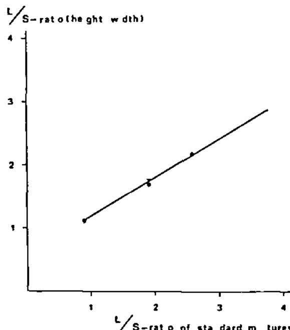



*Fig 2* Lecithin/sphingomyelin ratios of standard mixtures measured by height×width ratio on thin layer plates Each point is mean±S E of 13 determinations $r = 0.833$ The regression line was $Y = 0.600x + 0.606$ ($N = 52$)

equipment is needed it is simple to perform only small amounts of amniotic fluid are needed and the whole procedure takes relatively short time

## RESULTS

The centrifugation and subsequent filtration was found to remove a high percentage of the phospholipids (Table I) This occurred to a similar degree in amniotic fluids of two different gestational ages However this did not affect the phospholipid composition

The accuracy of height×width determinations of L/S ratio was tested with standard mixtures and amniotic fluid samples with known L/S ratios (determined by phosphorus determinations) The results are shown in Figs 2 and 3 There was a reasonably linear relationship between the height×width determinations of L/S ratio and the true ratios up to true ratios of about 4–5 The slopes of the regression lines for the data in figures 2 and 3 were similar and the correlation coefficients were 0 833 and 0 860 respectively

*To establish the probable maximum and minimum* values for the L/S-ratio and dipalmitoyl lecithin concentrations we analysed more

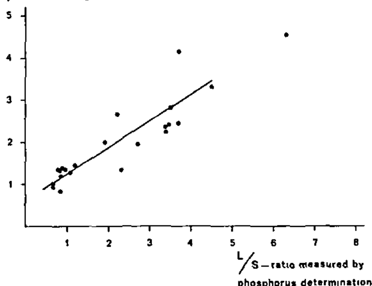



*Fig 3* Lecithin/sphingomyelin ratios of amniotic fluids measured by phosphorus determination and by height×width ratios on thin layer plates $r$=0 860 and the regression line was $Y$=0 6[illegible]8x+0 609 for all pairs with $X$<4.5 ($N$=33)

number of amniotic fluid samples from two different gestational ages and a surfactant rich fraction from adult human lung (Table II) The phospholipid composition was similar in amniotic fluid from the 40th week and in lung tissue in the sense that both were rich in dipalmitoyl lecithin and the L/S ratios were high Amniotic fluid from the 20th week contained considerably less dipalmitoyl lecithin and the L/S ratio was low The maximal ratios were roughly between 13–18 and the minimum values just below 1

With our method obviously only a part of this spectrum could be determined with any accuracy The concentration of phospholipid phosphorus in amniotic fluid (Fig 4) was low early in pregnancy but after the 35th week there was an increase

The distribution of phospholipid phosphorus among amniotic fluid phospholipids was determined in a number of samples (Figs 5 and 6) Lecithin and sphingomyelin amounted to 80–90 % of the total lipid phosphorus During gestation the percentage of

le II *Phospholipid composition and fatty acid composition of lecithin of a surface active fraction m human lung and of amniotic fluid from early pregnancy and at term*

| | Percent distribution of lipid phosphorus | Fatty acid composition (% by weight) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 14 0 | 16 0 | 16 1 | 18 0 | 18 1 | 18 2 | 20 4 |
| Amniotic fluid (20 weeks) | | | | | | | | |
| lecithin | 33 6 | 3 3 | 36 0 | 9 4 | 18 8 | 27 9 | 4 7 | – |
| sphingomyelin | 41 5 | | | | | | | |
| other | 24 9 | | | | | | | |
| Amniotic fluid (40 weeks) | | | | | | | | |
| lecithin | 80 9 | 3 4 | 65 3 | 14 5 | 6 3 | 7 8 | 2 3 | 0 4 |
| sphingomyelin | 4 6 | | | | | | | |
| other | 14 5 | | | | | | | |
| Lung[b] | | | | | | | | |
| lecithin | 72 4 | 6 4 | 68 0 | 13 5 | 3 7 | 7 2 | 0 8 | 0 4 |
| sphingomyelin | 5 4 | | | | | | | |
| other | 22 2 | | | | | | | |

Mean of two determinations
[b] Mean of three determinations
[c] 14 0=myristic acid 16 0=palmitic acid 16 1=palmitoleic acid 18 1=oleic acid 18 2=linoleic acid 18 3=linolenic acid 20 4=arachidonic acid

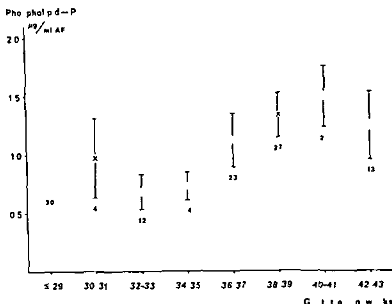



*Fig 4* Concentration of phospholipid phosphorus in amniotic fluid during gestation Each point is mean±S E of the number of determinations given in the *figure*

sphingomyelin fell from >45% to <20% The fraction of lecithin was around 40% early in pregnancy but increased rapidly after the 35th week up to just below 80% after 40 weeks Both the percentage of sphingomyelin and lecithin were significantly correlated to gestational age

The L/S ratio increased very slowly up to around the 35th week after which there was a vary rapid increase up to ratios around 3–4 at 40 weeks (Figs 7

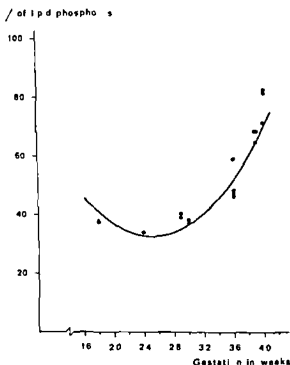



*Fig 5* Relative lecithin concentration in amniotic fluids of *varying gestational age Data from 47 individual samples* of amniotic fluid The curve $Y=134\ 9-8\ 18x+0\ 163x$ was fitted to the data with the aid of a computer program $r=0\ 764$

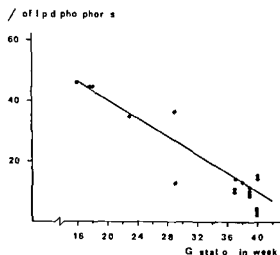



*Fig 6* Relative sphingomyelin concentration in amniotic *fluid of varying gestational age Data from 47 individual* samples of amniotic fluid The curve $Y=70\ 8-1.52x$ was fitted to the data with the aid of a computer program $r=-0\ 848$

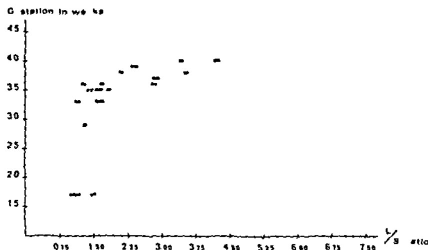


Fig 7 Scatter diagram of values for L/S ratio in amniotic fluid during gestation Each point represents one determination $r=0.712$ $P<0.0001$ $N=130$ $Y=5.20-0.382x+0.0085x^2$

and 8) The ratio was significantly correlated to gestational age (Fig 7) It is clear from figures 7 and 8 that the L/S ratio can be used for discrimination between gestational ages only between the 34th and 38th week as the curve is almost parallel with the axes above and below this interval The relation between gestational age and different borderline values for the L/S ratio is given in Table IV A

As amniotic fluid samples are sometimes contaminated with meconium it was considered important to find out if such contamination would influence the L/S ratio and phospholipid concentration An analysis showed that meconium (Table III) contained relatively small amounts of phospholipid The L/S ratio of the sample analysed was about 1 5 The dominating lipids were sterols free or esterified Heavy contamination with meconium might thus lower the L/S ratio after the 35th week

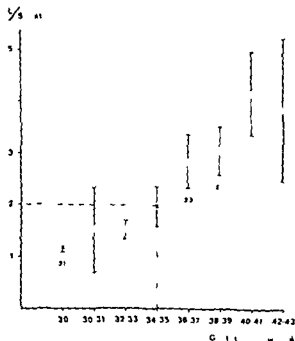


Fig 8 L/S ratio in amniotic fluid during gestation Each point represents the means of the number of determinations given in the figure The vertical bars indicate the 95% confidence interval for each mean

Creatinine concentration increased slowly from the 15th week up to about the 31st week after which the increase became somewhat more rapid The concentration of creatinine was significantly correlated to gestational age (Figs 9 and 10) The relation between gestational age and different borderline values for creatinine concentrations is given in Table IV B In Table IV C is shown the relation between gestational age and the combination of certain borderline values for L/S ratio and creatinine concentration

## DISCUSSION

A difficulty inherent in all determinations of gestational age is a lack of reliable data that might serve as references Many authors have used the weight or length of the newborn as the only reference or have based their calculations solely on the date of the last menstrual period (LMP) This might lead to severe errors The date of the LMP could be used provided regular periods preceeded the pregnancy The estimation of gestational age would be more correct still if the size of the pregnant uterus (examined before the 20th week) corresponded to the period of amenorrhoea and if fetal movements were experi

Table III *Lipid composition of meconium*

Mg/g dry weight Dry weight was 23% of wet weight Data from a sample from a 40-week-old fetus Each value is the mean of duplicate analyses

| | Lipid concentration (mg/g dry weight) | Percent distribution of lipid phosphorus | Fatty acid composition (% by weight) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 14 0 | 16 0 | 16 1 | –f | 18 1 | 18 2 | 18 3 |
| Phospholipid | 2 4 | | | | | | | | |
| lecithin | | 37 4 | 3 5 | 57 6 | 11 4 | 2 2 | 9 5 | 12 3 | 3 3 |
| sphingomyelin | | 22 8 | | | | | | | |
| lysolecithin | | 39 8 | | | | | | | |
| Free sterol[b] | 34 | | | | | | | | |
| Sterol ester[b] | 17 | | | | | | | | |
| Fatty acid ester[c] | 3 6 | | | | | | | | |
| Fatty acid[d] | 0 06 | | | | | | | | |

25×mg lipid phosphorus
[b] Calculated as cholesterol and cholesteryl palmitate
Calculated as tripalmitin after subtraction of ester bonds in phospholipid and sterol ester
[d] Calculated as palmitic acid
See footnote to Table II
[f] Not identified

enced by the woman at the appropriate time (22) Even so there will be an unavoidable scattering of ±2 weeks but the above criteria together constitute the best available guarantee that the estimated gestational age is correct and thus form a reliable reference Therefore the data used for comparison in this investigation can be considered valid

Another point of interest in investigations concerning the development of different parameters during pregnancy is the distribution of the material rather few observations usually being made in the interval 20–36th week of gestation The same applies partly to the present series but there are probably enough observations from the intervening weeks to make the whole series adequate for calculations

It is obvious that determinations of absolute values of phospholipid concentrations are strongly dependent on the rate of centrifugation and on whether filtration is performed or not Still our data on the absolute concentration of phospholipid in amniotic fluid (Fig 4) are not much different from those of others (29 32 44 50) Although considerable amounts of phospholipids are lost by centrifugation and filtration this does probably not change the L/S ratio (43 47 Table I)

The correlation coefficient (linear) between

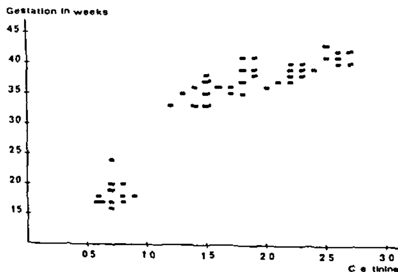



*Fig 9* Scatter diagram of values for creatinine concentration in amniotic fluid during gestation Each point represents one determination
$r=0.886$ ($p<0.0001$) $N=165$
$Y=1.12-0.065x+0.0023x^2$

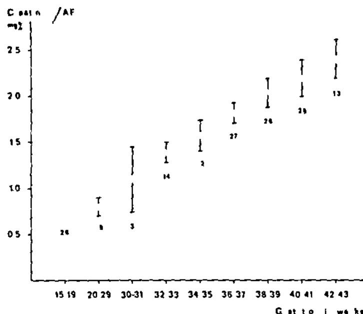



*Fig 10* Creatinine concentration (mg%) in amniotic fluid during gestation Each point represents the means of the number of determinations given in the figure The vertical bars indicate the 95% confidence interval for each mean

true ratios and ratios determined with the sliding caliper was 0 83 for standard mixtures of lecithin and sphingomyelin and 0 86 for authentic amniotic fluid samples (Figs 2 and 3) which are figures very similar to those obtained by Roux et al (50) The precision of the method for L/S ratio determination used in report is therefore reasonably good The most limitation of the method is its lack of precision at true L/S ratios larger than 4–5 which makes it a less sensitive method for determining the high ratios of gestational ages of more than 35 weeks (32)

The phospholipids and fatty acid composition of lecithin were very similar in the surfactant rich fraction from human lung and the amniotic fluid from the 40th week while the amniotic fluid from the 20th week differed significantly from this pattern The data are thus in accord with the view that the phospholipids that accumulate in the amniotic fluid late in pregnancy derive from the fetal lung (5 52)

Meconium (Table III) is sometimes a contaminant of amniotic fluid and it is thus of importance to know how it may influence the L/S ratio We found in the sample we analysed (obtained from a 40 week-old fetus) a rather low L/S ratio and a high percentage of lysolecithin We were thus unable to confirm the high L/S ratio of Kulkarni et al (39) Our data indicate that contamination with meconium might affect the L/S ratio mainly after the 35th week

We did not test the effect of blood on the L/S ratio There are however relevant data on human plasma phospholipids in the literature (45) from which it can be calculated that the L/S ratio in plasma is about 4 and that plasma thus may affect the ratio mainly

Table IV A *Gestational age (w) and L/S ratio in normal pregnancy*

| w | $N_I$ | L/S | $n_I$ | $n_I/N_I$ (%) | L/S | $n_{II}$ | w | $N_{II}$ | $N_{II}/n_{II}$ (%) |
|---|---|---|---|---|---|---|---|---|---|
| ≥36 | 65 | ≥1 85 | 57 | 88 | ≥1 85 | 65 | <36 | 8 | 12 |
| ≥36 | 65 | ≥2 25 | 49 | 75 | ≥2 25 | 53 | <36 | 4 | 8 |
| ≥38 | 42 | ≥2 25 | 34 | 81 | ≥2 25 | 53 | <38 | [1] 19 | [1] 36 |

$N$ = number of samples fulfilling the criterion of weeks
$n$ = number of samples fulfilling the criterion of L/S ratio
[1] case in the 34th week the rest older

Table IV B *Gestational age (w) and creatinine (Cr mg %) in amniotic fluid in normal pregnancy*

| w | $N_I$ | Cr | $n_I$ | $n_I/N_I$ (%) | Cr | $n_{II}$ | w | $N_{II}$ | $N_{II}/n_{II}$ (%) |
|---|---|---|---|---|---|---|---|---|---|
| ≥36 | 103 | ≥1 5 | 101 | 98 | ≥1 5 | 116 | <36 | 15 | 13 |
| ≥36 | 103 | ≥1 6 | 94 | 91 | ≥1 6 | 103 | <36 | 9 | 9 |
| ≥37 | 89 | ≥1 8 | 75 | 84 | ≥1 8 | 86 | <37 | 11* | 13 |
| ≥38 | 75 | ≥1 8 | 65 | 86 | ≥1 8 | 86 | <38 | 21* | 24 |
| ≥38 | 75 | ≥2 0 | 49 | 65 | ≥2 0 | 61 | <38 | 12 | 20 |

N = number of samples fulfilling the criterion of weeks
n = number of samples fulfilling the criterion of creatinine concentration
The remaining 10 cases had Cr ≥1 5 mg%
* 5 cases in the 35th week the rest older

before the 35th week This is in accord with the findings of Harding et al (32)

*The relative amounts of lecithin and sphingo* myelin at different gestational ages (Figs 5 and 6) agree well with the data of others (29 44 50) It should be noted that the percentage of lecithin in creased rapidly at about the same time as total lipid phosphorus (Fig 4) while there was no abrupt change in the percentage of sphingomyelin con comitant with the rise in lipid phosphorus suggesting that the phospholipids entering the amniotic fluid after the 35th week contained very little sphingo-myelin

Concerning the L/S ratio the main interest has been focused on the possibility of using this as an indicator of the maturation of the fetal lung and thus predicting a possible RDS (14 16 19 27 28 29 32 42 53 55) Less interest has been given its use in the estimation of gestational age in general (47 50) Gluck et al (29) and subsequently many others (4 14 16 27 55) found a significant rise in the L/S ratio beginning in the 34th–35th week and reaching maximal levels at term Nakamura et al (42) claimed that the L/S ratio is a reliable indicator of gestational age but not of pulmonary maturity In normal preg

Table IV C *Gestational age (w) and the combination of creatinine (Cr mg%) and L/S ratio in amniotic fluid*

| w | N | Cr ≥1 8 *and* L/S ≥2 25 (%) | Either C≥1 8 *or* L/S≥2 25 (%) | Cr <1 8 *and* L/S<2 25 (%) |
|---|---|---|---|---|
| ≥38 | 47 | 71 | 27 | 2 |
| 30–37 | 54 | 17 | 30 | 53 |
| 30–35 | 79 | 0 | 21 | 79 |

N = number of patients

nancies the L/S ratio has been found to correlate well to gestational age but not in high risk pregnancy with *maternal disorders (28 30) The change in* L/S ratio with increasing gestational age (Figs 7 and 8) agrees well with previous investigations (14 16 27 32 55) The absolute values for the L/S ratios are similar to those of Spellacy & Buhi (55) and others who use a similar technique but differs from the ratios reported by Quinlivan et al (47) As the absolute values for the L/S ratio de pend on the technique employed the data are not comparable

The usefulness of L/S-ratio in determining gesta tional age was fairly good within the interval be tween the 34th and the 38th week as illustrated by the finding that if the L/S ratio is ≥2 25 64% of the pregnancies were in the 38th week or more and 92% were in the 36th week or more At gestational ages less than the 35th week or more than the 38th week the usefulness of the *L/S ratio became less* which is a natural consequence of the appearance of the curve (Figs 7 and 8)

*The critical level of creatinine* indicating gesta tional age ≥ the 38th week has been discussed Several authors (17 48) state that a creatinine con centration of 1 7–2 0 mg% corresponds to a gesta tional age of at least 37 weeks Still others (40 46 49) find a good linear correlation between creatinine concentration and gestational age in normal preg nancy but not in cases of toxemia or intrauterine growth retardation

Harrison (33) obtained in 111 samples a 91% pick up rate (≥38 weeks) using a discriminating level of ≥2 mg% but only 5 cases had a gestational age <38 weeks but >32 weeks Others (35 58) find only 5% false positive creatinine values using 2 mg% as an indicator of a gestational age of >37 weeks The pick up rates were however only 40% and 53%

respectively and the false negative values showed a great scattering down to 1 1 mg% which is not the case in our material (Fig 9) According to Foulds et al (24) creatinine concentration is an unreliable index of gestational age since half of the values at 42 weeks fell within the range of 32 weeks Gauthier (26) presents in a series of 139 samples a pick up rate of 94% in the 36th week or more if creatinine was ≥1 6 mg% and a rate of 82% in the 38th week or more if creatinine was ≥1 8 mg% which corresponds very well with our data

We found that a creatinine concentration of ≥1 8 mg% predicts a gestational age of the 38th week in 75% of remaining 25% no case was less than in the 35th week Our data showed that if a creatinine level of 2 0 mg% was taken as maturity index the precision of prediction became less accurate without lowering the false positive rate (Table IV B)

The method used in this investigation for the determination of creatinine concentration in the amniotic fluid is roughly the same as has been used by most previous workers which makes comparisons between different results possible In our cases it is clear (Fig 9) that there is a wide scattering of values at each observation point e g at the critical point of the 35th week where the range is 1 2–2 2 mg% On the other hand a creatinine concentration of 1 8 mg% proved a suitable dividing line the gestational age in all cases being in the 35th week or more This is a lower figure than has been given by y other authors One explanation for this might that in most investigations the means have been used and not the 100% borderline Besides this there are several factors influencing the creatinine concentration in amniotic fluid such as fetal muscular development and renal function maternal serum creatinine concentration and other factors affecting the production and volume of amniotic fluid Considering these facts it seems not surprising that varying results are found However we think that determinations of the creatinine concentration in amniotic fluid is a good method for discriminating between gestational ages in the period between the 35th and 38th week

If only the L/S ratio of ≥2 25 was considered the gestational length was at least in the 34th week which is a time when the fetus has sometimes achieved lung maturity If only the creatinine concentration ≥1 8 mg% was considered all cases were at least in the 35th week Out of 42 cases which fulfilled the criteria of a L/S ratio ≥2 25 and a creatinine concentration of ≥1 8 mg% 71% were at least in the 38th week and the remaining 29% were all in the 36th or 37th week When L/S ratio and creatinine concentration are combined an increased possibility to discriminate between gestational ages <36th week and ≥38th week is obtained The combination thus increases the precision in determining the gestational age with 1–2 weeks a fact that is of great importance from a clinical point of view

## ACKNOWLEDGEMENTS

Supported by grant 13X 3364 from The Swedish Medical Research Council and grants from The Perinatal Research Foundation of the Expressen and from Ogra duerade Forskares fond from the University of Umeå The skilful technical assistance of Mrs Kerstin Thorzell and Mrs Monica Isaksson is gratefully acknowledged

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*Submitted for publication May 16 1974*

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Acta Obstet Gynecol Scand 54 437–442 1975

# SERUM ALKALINE PHOSPHATASE IN PREGNANCY

## I A Comparative Study of Total L Phenylanine sensitive and Heat Stable Alkaline Phosphatase at 56 C and 65 C in Normal Pregnancy

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*Abstract* 312 paired serial estimations of total L-phenylalanine sensitive and heat stable alkaline phosphatase (at two temperatures 56°C and 65°C) were performed on sera from 31 pregnant healthy women from 18 weeks to term Heat-stable alkaline phosphatase determined at 65 C enriched the serum in a smooth exponential course throughout the second and third trimester whereas the other enzymic activities investigated showed more irregular increments There was a slight but significant correlation between total alkaline phosphatase activity of the placenta and serum heat stable alkaline phosphatase at term whereas there was no correlation between the weight of the child at birth and heat stable alkaline phosphatase in the serum of the mother It is concluded that to test the feto-maternal unit determination of serum alkaline phosphatase should be performed at 65 C with due regard to appropriate sampling and experimental design

The alkaline phosphatase of the human placenta is a glycoprotein enzyme of known amino acid composition (19) The enzyme is produced by the placental trophoblast (30 31) and it differs from that produced by other organs in a number of physico-chemical characteristics including substrate specificity heat stability inhibition by amino acids antigenic properties electrophoretic mobility etc (1 2 8 9 17 28 30 31) It appears in the maternal circulation during the first trimester of gestation and persists in the serum up to 12 weeks after delivery (33) The enzyme is controlled by the fetal genotype and shows electrophoretic polymorphism determined by the occurrence of three common alleles and several less frequently occurring alleles at an autosomal locus (8 30 31)

Recent observations have suggested that placental alkaline phosphatase may play an important role in the feto-maternal immunological relationship (5 6 7) The increase of placental alkaline phosphatase activity in maternal serum follows an exponential course as a function of gestation time (18) and this has been taken to represent a serum index of a normal functioning placenta (21) So far however there is no unanimity as to the usefulness of serum alkaline phosphatase determinations in obstetric practice (3 4 12 15 21 22 24 34)

Except for the recently developed radioimmunoassay techniques (23 25) no specific method for the quantitation of serum placental alkaline phosphatase exists Thus in most studies placental phosphatase has been assayed indirectly by determination of the heat stable alkaline phosphatase advantage being taken of the heat stability of the placental isoenzyme In the majority of studies the temperature of inactivation has ranged from 55 to 65°C and the incubation time has varied from 5 to 60 min (for review see ref 18) making interpretation of the data rather difficult in terms of placental isoenzyme Evaluation is made still more complicated by the use of different substrates suboptimal pH and incubation conditions

Another approach is to determine placental phosphatase as enzyme activity inhibited by L phenylalanine L-tryptophan or L homoarginine (17) The inhibition by these compounds is stereospecific and it involves confirmational changes of the phosphoryl phosphatase upon binding of the amino acids thereby preventing dephosphorylation (10) The placental isoenzyme is inhibited by 79% whereas the isoenzymes from liver bone and intestine are inhibited approximately 8 10 and 80%

respectively (16) Hitherto these inhibitors have found little application in clinical chemistry

The present paper is part of a study designed to assess the usefulness of serum alkaline phosphatase determinations in obstetric practice and it deals with data from serial determinations of total (TAP) L-phenylalanine sensitive (LPSAP) and heat stable alkaline phosphatase (at two temperatures $HSAP_{56°C}$ and $HSAP_{65°C}$) made in the course of normal pregnancies

## MATERIALS

The study comprises sera and placenta from 31 healthy women (mean age 26 1/2 years range 19–36) attending the Outpatient Clinic Department of Obstetrics and Gynecology University of Bergen Women with diabetes hypertension or other internal diseases were not accepted For acceptance as normal pregnancy the following criteria were used

1 No urinary disease (sterile urine Labstix[1] negative)
2 Blood pressure <140/90
3 No Rh-immunization
4 Weight gain between 9–15 kg
5 No vaginal bleeding
6 Hemoglobin not less than 10 4 g/100 ml on at least three occasions during the third trimester
7 An uncomplicated vaginal delivery of a baby of normal weight (5th to 95th percentile) between 38 and 42 weeks without evidence of fetal hypoxia during labour or afterwards

In entering the study gestational age varied between 18 and 26 weeks The women were examined at regular intervals throughout pregnancy initially every third and fourth week and during the last trimester at weekly intervals

## METHODS

For enzyme analysis a fasting blood-sample was withdrawn If the specimen could not be processed at once the serum was frozen and stored at −20°C All determinations were run on a Technicon Auto-Analyzer as previously described (3?) Heat stable alkaline phosphatase was determined at two temperatures (i) 30 min at 56°C or (ii) 15 min at 65°C L-phenylalanine-sensitive alkaline phosphatase was determined according to Fishman et al (16)

At delivery the placenta (n=?2) was cut into pieces and rinsed with isotonic saline to remove blood blotted weighed and homogenized for 60 s in 4 vol of 0 25 M sucrose using a Waring Blendor setting 14 000 rpm The homogenate was centrifuged at 8 000 g for 30 min The supernatant was used as the source of crude enzyme A portion of the crude extract was subjected to butanol extraction 20% v/v for 15 min at 65°C The rest of the crude extract was incubated for 15 min at 65°C without any addition Extraction at 65°C was chosen to inactivate all non placental alkaline phosphatase (18) Both portions were centrifuged at 20 000 g for 30 min The aqueous supernatants were used for further analysis All operations were carried out at 0–4°C

Protein was determined as described by Eggstein & Kreutz (13)

[1] Labstix Ames Company Division of Miles Laboratories Ltd Elkart Ind

## RESULTS

The results are shown in Figs 1–3 All values are expressed as U/l From 5 to 12 samples were taken from each subject during the study

Fig 1 A–D shows the activities of the individual serum level of total alkaline phosphatase and the other modifications used in the course of normal pregnancies The lines represent the means of the individual observations along with the upper and lower limits including 95% of the results (along with the 2.5 and 97.5 percentiles) Starting with the 24–26th week the mean values rise progressively in all fractions The ranges however are seen to vary both within fractions and between fractions in the course of pregnancy The scatter is most pronounced with total alkaline phosphatase and least with $HSAP_{65°C}$ whereas $HSAP_{56°C}$ and LPSAP hold an intermediate position $HSAP_{65°C}$ enriches the serum in a smooth exponental course throughout the second and third trimester whereas $HSAP_{56°C}$ TAP and LPSAP show more irregular increments At term the means and ranges (±2 S D ) are 100±50 70±40 60±35 and 50±25 U/l for TAP LPSAP $HSAP_{56°C}$ and $HSAP_{65°C}$ respectively

In a number of studies HSAP was assayed at 55–56°C (11 12 24 34) while other investigators claim that this temperature is sufficient to inactivate all non-placental alkaline phosphatase (18 21 22)

The relationship between $HSAP_{56°C}$ and $HSAP_{65°C}$ is shown in Fig 2 There is a fairly close correlation between the results from the two assays the coefficient of correlation being 0 92 and the regression line $Y=1\ 2X+2\ 6$ where $Y=HSAP_{56°C}$ and $X=HSAP_{65°C}$

The relationship between $HSAP_{65°C}$ and weight of the child at birth is shown in Fig 3 A For babies weighing 3 200–4 200 g there is no correlation between weight and $HSAP_{65°C}$ at term (or any other of the other alkaline phosphatase fractions tested) On the other hand there is a slight but significant relationship between $HSAP_{65°C}$ at term and butanol extracted activity of alkaline phosphatase recovered

*Fig 1* Serum alkaline phosphatase in normal pregnancy The continuous line represents the mean values and the shaded area the normal range (2 5–97 5 percentiles) (A) Total alkaline phosphatase (B) L phenylalanine sensitive alkaline phosphatase (C) Heat stable alkaline phosphatase at 56 C (D) Heat stable alkaline phosphatase at 65 C

from the placenta (Fig 3B) The correlation coefficient is found to be 0 53 and the regression line $Y=0.002X+7$ where $Y$ represents $HSAP_{65°C}$ and $X$ total butanol-extracted alkaline phosphatase activity of the placenta

## DISCUSSION

In their initial studies of heat stable alkaline phosphatase Neal et al (28) incubated the specimens at 56°C for 15 min According to Fishman et al (16 18) this procedure is not sufficient to inactivate all non placental phosphatase At 65 C however non placental phosphatase are rapidly inactivated whereas the placental isoenzyme remains unaltered for at least 15 min (18) According to Hunter (21) failure to incubate at the correct temperature may result in a 20–35% contribution to the placental phosphatase of enzyme from sources other than the placenta thereby making interpretation of the results more difficult Our results vary slightly with those of Hunter (21) Thus on the average at term $HSAP_{56°C}$ exceeded $HSAP_{65°C}$ by 20% and from Fig 2 it can be seen that there was a fairly good correlation between $HSAP_{56°C}$ and $HSAP_{65°C}$ In our opinion the rationale for selecting 65 C instead of 56 C is limited not to the higher activity at the lower temperature but to irregularities observed with $HSAP_{56°C}$

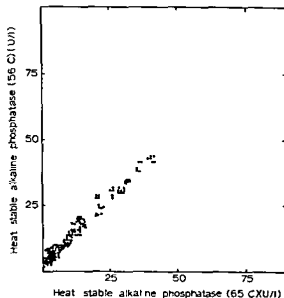
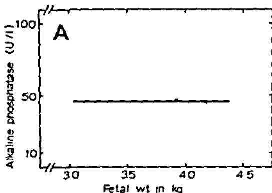



*Fig 2* Relationship between serum heat stable alkaline phosphatase activities determined at 56 and 65°C



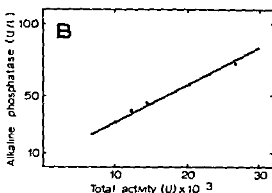




*Fig 3* (A) Relationship between fetal weight and serum heat stable alkaline phosphatase activity (65 C) at term (B) Relationship between total alkaline phosphatase activity recovered from butanol-extracted placentas and serum heat stable alkaline phosphatase activity (65°C) at term

and the variable presence of non placental isoenzymes Thus compared to $HSAP_{65°C}$ $HSAP_{56°C}$ is a less accurate indicator of minor changes in the serum level of placental phosphatase

To our knowledge extensive serial determinations of LPSAP in normal pregnancies are lacking In non pregnant women LPSAP represents mainly intestinal alkaline phosphatase whereas in pregnancy the placenta contributes to the serum LPSAP activity through the same mechanism as HSAP Theoretically therefore LPSAP should be equal to HSAP as a test of placental function However by comparing $HSAP_{65°C}$ and LPSAP a far wider scatter is observed with LPSAP both individually and throughout pregnancy These results could be explained by fluctuations in the residual LPSAP activity mainly from the intestinal isoenzyme (16) To minimize contribution from the intestinal isoenzyme however all specimens were taken from fasting subjects (27) Thus sources other than placenta and intestine must contribute to the serum LPSAP activity As a diagnostic tool then LPSAP is inferior to $HSAP_{65°C}$ for the following reasons (i) L phenylalanine is not a specific inhibitor of placental phosphatase isoenzymes from liver bone and intestine are likewise inhibited although to different degrees (26) (ii) the normal range of LPSAP is wider than that of $HSAP_{65°C}$ (iii) zig zag increments of LPSAP activity were regularly seen in contradiction to the smooth rise of $HSAP_{65°C}$ activity

As far as TAP is concerned we are dealing here with isoenzymes from various sources partly of known and partly of unknown origin This explains the poor correlation between TAP and HSAP the fairly broad normal range the irregular progress curves all findings reducing the usefulness of TAP in obstetrics

The lack of correlation between $HSAP_{65°C}$ and fetal weight is in agreement with results from other laboratories (14 29) However as the weights of all the babies lay between the 25 and the 75 percentiles the present study does not allow one to draw conclusions as to the value of $HSAP_{65°C}$ in prediction of small for date babies Conversely the results of this study provide no arguments against the possibility that an inverse correlation exists between birth weight and HSAP as claimed by Iyengar et al (24)

As far as the relationship between HSAP and enzymic activity of the placenta is concerned no unanimity exists (18 20 26) Thus contrary to the results reported in the present study Fishman et al (18) could find no relationship between $HSAP_{65°C}$ and the enzymic activity of the placenta From the results reported by Jeacock et al (26) that the enzyme activity of the placenta is related to the placental reserve it is tempting to suggest that $HSAP_{65°C}$ also reflects placental reserve The conclusion however is hampered by serious drawbacks since (i) the enzyme activity of placental homogenates determined *in vitro* can only give an indication of the potential activity of the enzyme *in vivo* and (ii) the amount of enzyme circulating in maternal blood as measured as $HSAP_{65°C}$ is not exclusively a function of the enzymic activity liberated from the placenta but is also determined by the maternal homeostatic mechanisms for controlling the blood enzyme levels

ACKNOWLEDGEMENT

The authors wish to thank Professor K Closs and Professor O Koller for their interest and advise throughout this study

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*Submitted for publication May 18 1974*

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Acta Obstet Gynecol Scand 54 443–448 1975

# ENZYMATIC STUDIES OF GLYCOGEN METABOLISM IN NONMALIGNANT AND MALIGNANT BIOPSIES FROM THE HUMAN UTERINE CERVIX

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*Abstract* The glycogen metabolism of the human uterine cervix has been investigated in tissue specimens from 147 women with gynaecological diseases 64 with cervical carcinomas 6 with carcinoma in situ and 77 with nonmalignant cervical diseases The glycogen content of the normal uterine cervix was found to be fairly constant and apparently not regulated by steroid hormones The low content of glycogen in malignant cervical tumours was confirmed by the present investigation The present investigation also showed that this abnormality was not only characteristic of invasive carcinoma but also of carcinoma in situ A particularly low glycogen content was found in tissue samples from patients with relapse Concerning the enzyme involved in glycogen metabolism a significantly high activity of glycogen synthetase was found in malignant cervical biopsies while the activity of glycogen phosphorylase did not differ significantly when comparing normal to malignant tissue

Most studies of the metabolism of glycogen in nonmalignant and malignant tissues from the uterine cervix have been based on histochemical techniques (for review see Nigam & Cantero in Cancer Research 1972 (16) and Langley & Crompton 1973 (13)

The presence of glycogen in the uterine cervix was first demonstrated by Schiller in 1933 (20) by the application of a dilute iodine solution to the cervical epithelium The absence of glycogen was believed to be an indication of malignancy However quantitative biochemical estimations of glycogen in human tissues have only been reported by a few authors (7 8 9 11 21)

The enzyme glycogen synthetase also referred to as UDPG glycogen transglucosylase [1] was discovered by Leloir & Cardini in 1957 (14) and is now recognized as the principle rate limiting enzyme in the pathway leading from glucose to glycogen (23) Two forms (I and D) of UDPG glycogen transglucosylase have been isolated The D form requires for its activity the presence of glucose-6-P while the I form is independent of this metabolite (6)

The breakdown of glycogen is a reversible process catalyzed by glucogen phosphorylase (Fig 1)

Considering the low content of glycogen in cervical tumours changes in the activity of the anabolic and catabolic enzymes of glycogen metabolism were to be expected However no quantitative studies of this type seem to have been made

The aim of the present investigation was to elucidate differences in glycogen metabolism between nonmalignant premalignant and malignant tissue samples from the human uterine cervix through quantitative studies of glycogen synthetase I and D and glycogen phosphorylase as well as of glycogen content An attempt was also made to correlate the biochemical findings with clinical stage and prognosis

## MATERIAL AND METHODS

The total material comprised cervical tissue specimens from 64 patients with invasive carcinomas 6 patients with precancerous lesions of the cervix and 77 women without any sign of malignant disease

The latter group served as a control group It was composed of 10 normal premenopausal volunteers 65 patients who had had a hysterectomy because of uterine

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Sponsored by the Danish Cancer Society

Abbreviations used AMP adenosine monophosphate EDTA ethylendiaminetetraacetate di sodium salt Pipes piperazine N N bis (2 ethane sulfonic acid) PPO–2,5 diphenyloxazole Tris HCl tris (hyroxymethyl)-aminomethane hydrochloride UDPG uridine 5-diphosphoglucose

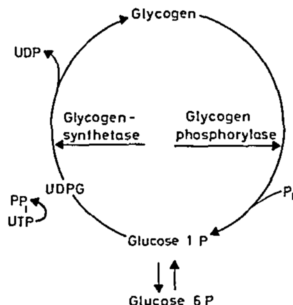



*Fig 1*

fibromyoma and 2 because of uterine retroversion The cervix in this group was histologically normal and without signs of infection

In the precancerous group the samples came from 6 patients operated by cone biopsy

The group of invasive carcinomas comprised 64 patients who were treated with radium and external irradiation The histological diagnosis was squamous carcinoma in all cases

Biopsies from the 10 normal volunteers were taken in the secretory phase The nonmalignant and precancerous specimens were taken at operation and the malignant ssues as biopsies from the tumour before treatment

Just after the tissue samples were taken from the pa tient one part was prepared for histology while the other one was frozen on dry ice transported to the laboratory and stored at −80°C until the experiments were perfomed Enzyme sudies were performed on samples taken from 19 patients without cancer and 23 patients with cancer of the uterine cervix Glycogen was determined in samples from 58 patients with nonmalignant disease 43 patients with malignancy and 6 patients with precancerous conditions In 3 patients both enzyme studies and glycogen determina tions were carried out

Table I *Glycogen content (normal)*

| | Glycogen |
|---|---|
| Proliferative | 9 10±2 50 (19) |
| Secretory | 6 16±2 50 (15) |

Units mg/g wet weight expressed in mean values ±2e ($s/\sqrt{N}$) In brackets number of patients

Table II *Glycogen content (normal)*

| Age | Glycogen |
|---|---|
| 20–30 | 7 18±3 00 (13) |
| 30–40 | 8 82±2 64 (16) |
| 40–50 | 7 01±1 84 (19) |
| >50 | 9 72±3 16 (10) |

Units mg/g wet weight expressed in mean values±2e ($s/\sqrt{N}$) In brackets number of patients

## ENZYME DETERMINATIONS

The tissue samples for enzyme studies were homogenized in Tris EDTA buffer (pH 7 8) first in a Ultraturrax homogenizer then in a glass homogenizer with Teflon pestile Then the homogenate was centrifuged for half an hour 20000 *x* at a temperature of 4°C The pellet was discarded and the supernatant was used for enzyme assay

Enzyme assay of phosphorylase Phosphorylase activ ity was determined as described by Wang & Esmann (24) The method utilizes the ability of phosphorylase to catalyze the incorporation of ¹ C labelled glucose from ¹⁴C glucose 1 phosphate into glycogen in the reversible process of glycogen catabolism (Fig 1) The glycogen thus formed is precipitated on filter paper by immersion into ice cold ethanol

An assay mixture with 100 mM glucose ¹ C 1 phosphate with a specific activity of 55000 DPM/μmole 1.5% glycogen and 100 mM KF was prepared in 100 mM PIPES buffer pH 6 4 AMP was added to yield a final concentra tion of 1 5 mM

Aliquots of 60 μl of the assay mixture and 30 μl of the supernatant were pipetted into disposable test tubes mixed carefully and incubated for 10 minutes at 30°C in a shaking water bath

At the end of incubation 75 μl of the reaction mixture were withdrawn and spotted on 2×2 cm squares of What man 31 ET filter paper The paper was immediately im- mersed into ice-cold 66% ethanol together with 6 blank filter papers prepared with assay mixture without enzyme

The filter paper squares were washed in 66% ethanol in a conical flask and stirred with a Teflon-covered magnetic stirrer The ethanol was changed several times till the blank filter papers showed activities below 40–60 DPM The paper squares then were rinsed in acetone and dried under infra red light and counted in plastic scintillation vials containing 10 ml 0.5% PPO in toluene

The counting efficiency was estimated by adding known volumes of the assay mixture without enzyme to Whatman square filters After drying they were counted with the series of assays No radioactivity was found in the vials after the filter papers were removed The results were corrected for counting efficiency and background and ex pressed in nanomoles per mg protein per hour

The protein content of the supernatant was determined in accordance to the method of Lowry et al (15)

Enzyme assay of glycogen synthetase The assay of glycogen synthetase was a modification of the method described by Thomas et al (22) The activity of syn

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assay mixture
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mM) pH 6 8 containing
proximately 200 000) DPM/
presence or absence of 10

l were added to 60 μl of the
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s at 100°C in a water bath
glycogen precipitated with
cordance to the method of

The glycogen was hydrolyzed by adding 0 6 N HCL and heated for 2½ hours in a water bath

After cooling glycogen was estimated as glucose by the hexokinase/glucose-6-phosphate dehydrogenase method (19)

By means of a converting factor the values were expressed as mg glycogen per g wet weight.

Table IV *Glycogen content—clinical stage*

| Stage I | Stage II | Stage III |
|---|---|---|
| 1 42±0 46 (15) | 0 99±0 28 (18) | 1 15±0 56 (8) |

Units mg/g wet weight expressed in mean values ±2e ($s/\sqrt{N}$) In brackets number of patients

Table V *Glycogen content (malignant)*

| | Glycogen |
|---|---|
| Premenopausal | 1 31±0 36 (23) |
| Postmenopausal | 1 04±0 28 (18) |

Units mg/g wet weight expressed in mean values ±2e ($s/\sqrt{N}$) In brackets number of patients

Statistical analysis was carried out according to the Wilcoxon rank test (7) if not otherwise stated

## RESULTS

The mean concentration of glycogen in normal samples from the uterine cervix of 58 patients was found to be 8 01 mg per gram of wet weight of tissue No significant difference was found between samples of tissue in the proliferative and in the secretory phase (Table I) and between samples obtained from various age groups (Table II)

The low content of glycogen in malignant tumours of the human uterine cervix was confirmed by the present investigation From Table III it appears that the glycogen concentration per gr wet weight of tumour tissue was 6–7 times lower than that of normal cervical tissue By the Student s test this decrease was significant with a $p$ value of $<0\ 001$ The present investigation also showed that this abnormality was not only characteristic of invasive carcinoma but also of carcinoma in situ ($p<0\ 01$)

The glycogen content of the premalignant tissue did not differ significantly from that of invasive carcinoma as a whole and no significant differences could be demonstrated between the various clinical stages of the malignant tumour (Table IV)

Comparisons of the glycogen concentration in invasive carcinomas from pre and post menopausal patients and in the different age groups did not reveal any significant differences (Tables V and VI)

Table VI *Glycogen content (malignant)*

| Age | Glycogen |
|---|---|
| 20–30 | 0 58±0 57 (3) |
| 30–40 | 1.57±1 50 (4) |
| 40–50 | 1 39±0 36 (16) |
| >50 | 1 02±0 27 (18) |

Units mg/g wet weight expressed in mean values ±2e ($s/\sqrt{N}$) In brackets number of patients

Table VII *Glycogen content—survival*

| | Glycogen |
|---|---|
| Alive | 1 34±0 28 (30) |
| | P=0 05 |
| Dead or recurrence | 0 75±0 40 (11) |

Units mg/g wet weight expressed in mean values ±2e (s/√N) In brackets number of patients

The observation period in the present investigation was 17–18 months At that time 30 patients were alive without any sign of recurrence while 11 patients had relapsed or died In the latter group 5 were dead while 6 were alive with signs of progressive disease *From Table VII it appears that the* mean glycogen concentration of tissue samples from the former group was almost double that of the latter This difference was at the borderline of statistical significance

The low glycogen content of invasive carcinoma of the cervix could not be ascribed to a low concentration of glycogen synthetase On the contrary *from Table VIII* it appears that both synthetase I and D showed significantly higher vaues in malignant tissue samples than in normal samples On the other hand no significant differences in phosphorylase activity could be demonstrated between the two groups (Table VIII)

The enzyme values differed too much to allow the establishment of any significant correlation between clinical stage and enzyme activity in the resent material (Table IX)

Table X suggests that high enzyme activities correlate *with a poor prognosis However the differ*ences shown in the table between patients alive without signs of recurrence and patients dead or alive with relapse were not statistically significant

DISCUSSION

*The present quantitative data as well as observa*tions by others (7 9) demonstrate that the glycogen content of the normal uterine cervix is fairly constant independent of age and apparently not regulated by steroid hormones By contrast glycogen synthesis of the human uterine endometrium is under hormonal influence exhibiting cyclical variations with the lowest glycogen content during the proliferative phase and the highest during the secretory phase (1 9 11)

The lack of quantitative changes in glycogen levels of the uterine cervix during the normal cycle is in agreement with the observations of other investigators (7)

Based on his histochemical studies Schiller (20) concluded that the absence of glycogen *in* human cervical tissue is a sign of malignancy This was contradicted by Foraker et al (4) who found a positive histochemical glycogen reaction in more than 50% of the biopsies examined from patients with cervical cancer These authors suggested that the amount of glycogen depended on the rate of differentiation Biochemical studies by Gregoire et al *(9) did not reveal significantly lower* values of glycogen in ectocervical tissue from patients with cervical cancer but the series was small (6 patients) and it was not stated in which part of the cervix the tumour was localized

In the present study glycogen was found both in biopsies from patients with carcinoma in situ and patients with invasive cancer However the content of glycogen was significantly lower compared to nonmalignant tissue

No difference could be demonstrated between the glycogen content of tissue samples showing carcinoma in situ and those showing invasive carcinoma and no significant *correlation between* clinical stage age and glycogen content was *established*

On the other hand the present results indicate that relapse and early death correlate with a particularly low glycogen content which may possibly *be taken as a sign of a high degree of malignancy* This observation also indicates that glycogen deter

Table VIII *Glycogen metabolism enzymes*

| | Synthetase I | Synthetase D | Phosphorylase |
|---|---|---|---|
| Normal | 8 06±2 96 (19) | 23 70±9 60 (19) | 747 82±286 10 (18) |
| | p<0 02 | p<0 01 | p<0 1 |
| Cancer | 22.54±11 90 (23) | 87 15±30 18 (23) | 830 04±314 14 (22) |

Units nmoles/mg protein/hour expressed in mean values ±2e (s/√N) In brackets number of patients

Table IX *Glycogen metabolism enzymes*

| | Synthetase I | Synthetase D | Phosphorylase |
|---|---|---|---|
| Stage I | 26 69±27 72 (5) | 76.52±64 84 (5) | 587 81±471 32 (5) |
| Stage II | 21 99±19 78 (12) | 72 46±27.52 (12) | 813 96±530 22 (12) |
| Stage III | 17 59±13 22 (5) | 138 33±14 22 (5) | 1261 16±1419 69 (4) |
| Stage IV | 33 08 (1) | 60 76 (1) | 509 67 (1) |

Units nmoles/mg protein/hour expressed in mean values $\pm 2e\ (s/\sqrt{N})$ In brackets number of patients

minations may be a useful prognostic tool but a longer observation period than the present 18 months should be allowed before final conclusions are drawn

An interesting observation was made by Gregoire et al (9) in the studies of glycogen content in patients with cervical cancer Even if the glycogen content did not differ significantly from normal in ectocervical cancer they observed a decreased glycogen content of the endometrium from the same patients They suggested that the presence of tumours may affect glycogen metabolism in regions apart from the actual localization of the lesion Accordingly in cervical epithelium from a patient with ovarian cancer we observed malignant values both of the enzymes involved and glycogen content The histological diagnosis of the cervix was normal This observation might be interpreted as a defect of superior hormonal control of glycogen metabolism However as already stated our comparative studies of proliferative and secretory tissues as well as premenopausal and postmenopausal tissues of the uterine cervix do not point to any hormonal regulation of glycogen metabolism

In view of the low glycogen content of cervical tumours the 6–7 fold increase of the activities of glycogen synthetase I and D observed in the present investigation was unexpected Increased cellularity could theoretically account for the increased enzyme concentration However if this were the case then one would expect a similar increase in phosphorylase activity But such an increase was not observed

As already mentioned most studies of glycogen metabolism in human cervical tissues have been performed with histochemical techniques This also applies to investigations of the enzymes involved Such investigations have been carried out by Fienberg et al (3) and by Foraker et al (5)

They showed that the enzymes involved in the synthesis of glycogen in the normal cervix are localized to the deeper epithelial layers in cells which are less differentiated compared with cells in the upper epithelial layers Against this background the results of the present quantitative studies of glycogen synthetase may be interpreted as a sign of cellular dedifferentiation The low glycogen content in spite of the high activity of anabolic enzymes might suggest an increased glycogen turnover However this assumption is not supported by the normal phosphorylase activity observed in the present investigation

Competition for necessary metabolites might be a better explanation In previous investigations in this laboratory (17 18) a two- to three fold increase in glucose consumption and lactate production was demonstrated in malignant biopsies from the uterine cervix In agreement with this observation a considerable increase was seen in the activities of hexokinase phosphofructokinase glucose-6-P dehydrogenase pyruvate kinase and lactate dehydrogenase This increase in glucose breakdown might result in lack of substrate for glycogen synthesis

From these results and considerations one may conclude that a low content of glycogen is an early

Table X *Glycogen metabolism enzymes survival*

| | Synthetase I | Synthetase D | Phosphorylase |
|---|---|---|---|
| Alive | 19 25±10 44 (15) | 81 42±3? 84 (16) | 602 86±196 84 (15) |
| | $p>0\ 10$ | $p>0\ 10$ | $p>0\ 10$ |
| Dead or recurrence | 28 69±29 02 (8) | 100 19±59 04 (8) | 1 316 86±814 28 (7) |

Units mg/g wet weight expressed in mean values $\pm 2e\ (s/\sqrt{N})$ In brackets number of patients

sign of malignancy in the uterine cervix The low glycogen content is not due to enzyme deficiency but may possibly be explained by lack of substrate due to the increased rate of glucose catabolism Suggestive evidence has been presented that low glycogen levels correlate with a poor prognosis

Glycogen synthetase I and D are increased in invasive carcinomas of the cervix while no changes could be demonstrated in the activity of phosphorylase The increased glycogen synthetase activity might be interpreted as a sign of cellular dedifferentiation

The author expresses thanks to Ellen Møllgaard and Tine Raunstrup Hansen for technical assistance

## ACKNOWLEDGEMENTS

This study was carried out with the support of the Daell Foundation and the Danish National Research Foundation

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*Submitted for publication May 28 1974*

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Acta Obstet Gynecol Scand 54 449–461 1975

# ON SOME LATE EFFECTS OF BILATERAL OOPHORECTOMY IN THE AGE RANGE 15–30 YEARS

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*Abstract* Between 1910 and 1940 146 young females aged 15–30 years underwent bilateral salpingo-oophorectomy as part of a radical operation for salpingo-oophoritis These women or their records were reviewed in 1971 42 women had died in the meantime More than half (22) of them had died from cardiovascular diseases 5 from carcinoma of the uterus and 4 had committed suicide None had died from carcinoma of the breast Of 68 who were still alive information by questionnaire was obtained and 32 were admitted to hospital for extensive examination 32 age matched women to be operated on for prolapse but with no other known disease of the reproductive tract served as controls A further control group was added as 11 of the 68 were found to have menstruated again after the operation which had evidently not completely removed the gonads Complete oophorectomy was found to have been followed by (*a*) an increased incidence of cardiac symptoms and nervous diseases as well as an increased use of drugs (*b*) a significant increase in the frequency of coronary vascular diseases in ages up to 70 years (*c*) an increase in the serum cholesterol and triglycerides most significantly in the ages below 60–65 years Women with symptomatic coronary disease had a higher serum cholesterol level than women without and women with signs of peripheral vascular diseases had a significantly higher concentration of serum triglycerides (*d*) an increased frequency of fractures (radius and femoral neck) increased osteoporosis and thinner cortical bone The brittleness of the skeleton was correlated with low excretion of oestrogens in the urine No vertebral compression or abnormal decrease in height was observed (*e*) an increased adrenocortical activity with significantly increased excretion of 17 ketosteroids 17-OH ketosteroids and low polar total oestrogens This activity abated in women above 65 years (*f*) a traumatic psychological experience of the accompanying sterility while sexuality seemed to be largely unaffected in many of them Almost half of the women examined by the psychiatrist were unusually mentally active and agile and they had a lower excretion of estriol than the rest

The effect of the ovaries on the panorama of diseases and causes of death of women has long attracted attention Most published investigations have been based on women oophorectomised for a variety of reasons

It is believed that bilateral oophorectomy has a favourable effect on several types of tumour mainly by the elimination of most of the oestrogenic hormones Such hormones are thought to play a role in the genesis and further growth of tumours of the reproductive tract and of the breast Oophorectomy has been tried also in the treatment of carcinoma of the breast

Bilateral oophorectomy with a substantial reduction of oestrogens is stated to be followed by an increased frequency of disease of the cardiovascular system with an increased tendency to arterial hypertension and arteriosclerosis and an accompanying tendency to coronary sclerosis (64 53) myocardial infarction (41) and cerebral haemorrhage (4 49) Symptoms of coronary disease are more common in women after oophorectomy (53) Coronary atheroma is more common in men than in women up to the age of 60 years after which the difference narrows This difference in incidence like the increase in the frequency of symptomatic coronary disease (CHD) after the menopause corroborates the assumption that the higher blood level of oestrogens in women with preserved gonadal function offers protection against the development of coronary atheroma It has also recently been shown that the menopause is more often premature in women who have had myocardial infarction than in the population in general (7)

The composition of lipids in the serum has received much space in the discussion of the development of arteriosclerosis over the last 20 years The

serum cholesterol level is much higher in women than in men during the sixth decade of life Oophorectomy prior to menopause raises the serum cholesterol level (3 42 43 53 58) Oestrogenic hormones evidently have a strong influence on the lipid and lipoprotein content of the blood (59) Oestrogens increase the phospholipid concentration suppress cholesterol raise the alpha lipoprotein level and depress the beta lipoprotein In women who have been oophorectomised or who are in the climacteric the lipid and lipoprotein pattern resembles that in men But treatment of such women with oestrogenic hormones will cause the levels of the above serum fractions to return to normal

Electrocardiographic changes are abnormally common after oophorectomy (43) but the frequency of such changes can be reduced by treatment with oestrogens (15) This lends further support to the idea that oestrogens protect women against cardiosclerosis

Various endocrine organs are affected by loss of ovarian function Such loss has been assumed to cause a generally increased hypophyseal activity with an increase not only of the production of gonadotropins but also of other hormones such as ACTH and TSH It has been claimed that the adrenal cortex can increase its activity in oophorectomised women and help to maintain the production of oestrogens It is possible that oophorectomy may be followed by disturbances of thyroid function by a tendency to diabetes and an increased frequency of obesity

The classical symptoms of loss of the ovaries (i e symptoms due to the loss of oestrogens) are various neurovegetative symptoms such as flushes Mental symptoms such as lack of initiative apathy and depression or increased activity restlessness or unrest may also occur In addition anxiety and psycho-sexual aberrations have been reported (46)

The almost complete loss of the production of sex hormones after oophorectomy seems to have an effect also on the cortex and trabeculae of the bones This results in osteoporosis with an increased risk of fracture after even relatively trivial trauma But osteoporosis is an insidious condition which is not easy to diagnose early The condition is not radiologically demonstrable until the skeleton has lost at least 30% of its calcium content i e not before the process is well advanced As early as 1941 Albright et al (1) postulated that the loss of endocrine function of the ovaries was a contributory cause of osteoporosis in women but this assertion was later denied by Donaldson & Nassim in 1954 (17) who stressed the importance of ageing Increased parathyroid activity due to loss of oestrogenic suppression might well explain the increased serum calcium and increased excretion of calcium in the urine as well as the negative calcium balance (65) But also increased serum phosphorus levels have been reported (1) which would rather suggest decreased parathyroid function

Oestrogens are excreted though in low concentration in the urine also after complete oophorectomy Such oestrogens as a rule <10 μg a day (16) are believed to originate mainly from the adrenals since administration of ACTH to oophorectomised women increases the excretion of oestrogens in the urine (11)

After the menopause the excretion of oestrogens in the urine is normally about 10 to 15 μg per day which means a rate of production that is no longer capable of stimulating the endometrium (12) A fall in the level of the oestrogens in the climacteric or after oophorectomy results in an increase in the blood and urine level of gonadotropins mainly of FSH but also of LH (44) The excretion of 17 ketosteroids 17 ketogenic steroids pregnanetriol testosterone and corticoids decreases in old age—the adrenopause—but does not appear to be correlated with ovarian function

The urogenital manifestations of loss of production of oestrogens include atrophy of the mucosa of the vagina urethra and bladder with decreased resistance to infections The uterus as well as any existing myoma becomes smaller and the endometrium becomes thin and inactive The cervix becomes less protuberant and the fornices are shallow

In many departments of gynaecology hysterectomy carried out for benign diseases of the reproductive tract is often extended to include routine oophorectomy especially in women of premenopausal or menopausal age in an endeavour to prevent the later development of ovarian tumours But such an operation however appears to be capable of leading to climacteric symptoms even in women after the menopause if the oestrogenic production by the ovaries exceeds 20 μ per 24 hours (39)

In earlier published series of oophorectomised women the majority were operated on late in the reproductive period of life (4)

Many women in the district covered by Malmö general hospital have been oophorectomised early in life, however. This is because early in the century advanced salpingitis was often treated surgically at our hospital. Not only the tubes but also the ovaries were radically removed. This surgical treatment, which was performed even on girls as young as 15 years of age, was abandoned with the advent of sulphonamides at the end of the 1930s.

This unique series was analysed in respect of morbidity, survival and causes of death. Those patients who were still alive and residing in the Malmo district were admitted to the hospital and investigated with regard to nervous disorders, cardiovascular status, osteoporosis and hormonal state.

## MATERIAL AND METHODS

The 1910–40 files of Malmo General Hospital were searched for the names of women operated on because of salpingo-oophoritis with salpingectomy and bilateral oophorectomy before the age of 30 years. With the help of the parish offices and registry offices most of the women still living could be traced. The entire series consisted of 146 women who had operations between the ages of 15 and 30 years (mean age 25 years). Of these women, 42 had died in the meantime. The causes of death were noted either from the death certificates or sometimes from the autopsy reports. Of the 104 survivals, who at the time of the present review in 1971 were on average 69 years old, 68 were living in the area served by Malmo General Hospital and were contacted. 32 (group A) of these women, aged 53–84 years, who had their operations at an average age of 24 years and who were living within the town were invited to a free gynaecological and physical examination. All were accepted and all were admitted to the hospital. The remaining 36 (group B) were sent a questionnaire which they were asked to fill in and return. All cooperated. As controls we used 32 randomly selected age matched women (group C) admitted to the department for surgical treatment of prolapse of the uterus but with no other disease of the reproductive organs.

Of the 68 living women who had been operated and who were still alive, 4 of group A and 7 of group B continued to menstruate after the operation, which had thus not removed all ovarian tissue. This group of incompletely treated women—group D ($D_A+D_B$)—thus constituted a second control group of 11 cases. In the tables the figures given for groups A and B do not include women belonging to group D.

The questions in the questionnaires (which were also posed to the patients admitted to hospital) concerned civil status, adopted children, later treatment at hospital (and if so, when, where and why), bone fractures, later medical examinations for heart symptoms, arterial hypertension, vessel cramp, nervous symptoms or any other disease.

The women admitted to hospital were examined routinely, besides which determinations were made of the total oestrogens according to Carlström et al (14), 17-ketosteroids with the method of Vestergaard (62) and Birke et al (8) and 17-OH ketosteroids in the way described by James et al (26) in 24-hour samples of the urine. Urine voided under sterile conditions was cultured for bacteria. At the gynaecological examination vaginal smears were obtained and examined for malignant cells as well as for the effect of oestrogens.

The thickness of the cortical bone of the proximal shaft of the radius was examined radiologically according to Meema (36) to assess the extent, if any, of osteoporosis. The serum calcium and phosphorous were also determined.

Out of the 32 women of group A, 20 were randomly selected and thoroughly examined psychiatrically on the basis of a 90 minute traditional psychiatric interview designed to cover relevant aspects of subjects' lives, with special emphasis on psychiatric and psychosexual factors. It would have been impossible to evaluate the controls blindly and as the estimated variables were to a large extent subjective, it was decided not to include the controls in the psychiatric part of the investigation.

The women were examined cardiologically for coronary disease and stenosing arterial disease of the legs. Determinations were also made of the serum lipids. In the analysis of the material two age limits were used, one at 75 years and one at 65 years.

A special search was always made for angina pectoris and intermittent claudication with the criteria published by WHO. A 12 lead electrocardiogram (ECG) was recorded and in most patients an exercise test was performed with the patient in the sitting position on an electrically braked variable load bicycle ergometer (Elema Schonander, Stockholm–Solna). The initial work load was 200 kpm per min and if a steady state was achieved within 4 minutes the load was increased to 400 kpm per min and thereafter, if possible, to 600 kpm per min. The ECG was recorded on a direct ink writing machine (Mingograph 81, Elema Schonander).

The following leads were recorded with the patient supine before exercise: I, II, III, aVR, aVL, aVF, V, $V_2$, $V_3$, V, $V_5$ and $V_7$.

During exercise the reference electrode was placed on the forehead and leads $CH_2$, CH, $CH_5$ and CH were recorded. CH leads were also recorded before and immediately after exercise while the patient was sitting on the bicycle, and 5 and 10 minutes respectively after exercise while the patient was resting supine. Two minutes after exercise with the patient resting supine a complete 12 lead electrocardiogram including V leads was made. The heart rate during exercise was, as a rule, calculated every minute from the ECG. The systolic blood pressure was measured every other minute and just before the end of exercise by the auscultatory method with sphygmomanometer cuff wrapped around the right arm. The respiration rate was measured with a stethoscope every other minute. In the analysis of the ECG only ST depressions of 1 mm or more (measured from the end of the *P R* segment) and horizontal or downward sloping ST segments were considered abnormal.

Working ECG was judged by two cardiologists independently of one another.

Table I *Survey of causes of deaths*

| Age (year) at death | Cancer | Cardio vascular disease | Tuberculosis | Violent death | Miscellaneous | Number |
|---|---|---|---|---|---|---|
| 25–30 | | | 1 | | | 1 |
| 31–35 | 1 | | 1 | | | 2 |
| 36–40 | | 1 | | 1 | | 2 |
| 41–45 | | 2 | 1 | | | 3 |
| 46–50 | 1 | | | 3 | | 4 |
| 51–55 | 3 | 3 | | | | 6 |
| 56–60 | 2 | | | | | 2 |
| 61–65 | 1 | 5 | | | 1 | 7 |
| 66–70 | | 1 | | | 1 | 2 |
| 71–75 | 1 | 4 | | 1 | | 6 |
| 76–80 | | 5 | | 1 | | 6 |
| 81–85 | | 1 | | | | 1 |
| Total | 9 (22%) | 22 (52%) | 3 (7%) | 6 (14%) | 2 (5%) | 42 |
| Mean age at death | 54 | 65 | 33 | 55 | 65 | |

Cardiac decompensation was classified according to the criteria of the New York Heart Association Heart volume with the patient standing was measured according to Jonsell (27) and relative heart volume i e ml per square meter body surface area (ml/m² BSA) was estimated from a radiograph according to Lysholm et al (33)

Serum triglycerides was determined by the method of Laurell (31) and the total cholesterol was measured in a Technicon Auto Analyzer (10 30 45)

Hypotheses were tested with Student s *t* test and calculation of $\chi^2$ sometimes with Yate s correction (57)

## RESULTS

*Causes of death* (42 cases)

The causes of death given in the death certificates and autopsy reports of the 42 women who died before our review are distributed among the main groups in Table I A list of those who died from carcinoma and from cardiovascular diseases is given in Table II

Table II *Causes of death*

| | Number of patients | Age (year) Mean age |
|---|---|---|
| *Cancer* | | |
| Bronchial carcinoma | 1 | 71 |
| Pharyngeal carcinoma | 1 | 64 |
| Carcinoma of the pancreas | 1 | 57 |
| Carcinoma of the cervix | 2 | 54 |
| Carcinoma of the uterine body | 3 | 44 |
| *Cardiovascular diseases* | | |
| VOC+cor incomp angina | 3 | 56 |
| Myocardial infarct | 3 | 57 |
| Cardiosclerosis | 4 | 69 |
| Cerebro-vascular accident | 5 | 68 |
| Arteriosclerosis | 3 | 66 |
| Arteriosclerosis + Parkinsonism | 1 | 74 |
| Pulmonary embolism | 3 | 75 |

In 22% of the 42 patients who died the cause of death was carcinoma The corresponding figure for the whole country in 1968 was roughly the same or 19 5% (60) It is noteworthy however that in 5 of the 9 cases the tumour was in the uterus which seems to be an over representation of this organ whereas there was not a single case of carcinoma of the breast Early oophorectomy thus appears to offer protection against carcinoma of the breast

The frequency of deaths from cardiovascular diseases especially cerebrovascular lesions myocardial infarction and cardiosclerosis was high suggesting that oophorectomy early in life accelerates development of atheroma

The frequency of death from tuberculosis however was 7% compared with 0 7% for the country as a whole in 1968 This difference can probably be explained by the marked decrease in the frequency of tuberculosis during recent years and by the fact that some of the patients operated on because of salpingitis had tuberculous salpingitis

The frequency of violent deaths was 14% (6 cases) compared with 6.5% for the whole country in 1968 Of the 6 patients as many as 4 had committed suicide (oophorectomy at 21 23 29 and 30 years) This means that almost 10% of the women without ovaries who died committed suicide which is a remarkably high figure The cumulative age and sex specific

Table III *Information obtained by questionnaire*

| | Group A+B | Group C | Group D |
|---|---|---|---|
| Number of patients | 57 | 32 | 11 |
| Married | 47 | 30 | 10 |
| Children before operation | 22 | 29 | 2 |
| Adopted children | 10 | 0 | 3 |
| | Group A+B (57) | Group C+D (43) | *P* |
| Biliary disorders | 20 | 11 | n s |
| Cardiac disorders | 23 | 8 | sign |
| Hypertension | 20 | 16 | n s |
| Intermittent claudication | 6 | 4 | n s |
| Nervous complaints | 22 | 7 | sign * |
| Fractures | 23 | 10 | n s |
| Various other disorders | 32 | 22 | n s |
| Receiving medication | 38 | 17 | sign |

risk of suicide during 1920–69 based upon the national incidence (9) was calculated as 0 64 for the entire sample The observed number 4 was thus more than 6 times that expected However it should be kept in mind that many of the patients had many social problems at time for operation As early as 1962 Ask Upmark (4) reported 2 cases of suicide among 38 deaths in a review of women who had both ovaries removed These women had however been operated on relatively late in life (at 40 and 45 years)

### *Survivors examined*

#### *Anamnestic data*

It was striking that many of the women had married after their operations and were evidently mentally and sexually adjusted to married life despite sterility absence of ovaries and menstruation (Table III) Many of them had adopted children

The control group in whom ovarian ablation had been incomplete (D) was so small that in further comparisons it was pooled with the group of controls

Table IV *Cardiovascular status Women ≤75 years*

| | Oophorectomised (*n*=41) | Controls (*n*=29) |
|---|---|---|
| Myocardial infarction | 3 | 0 |
| Angina pectoris | 8 | 3 |
| Pos working ECG not AP | 0 | 3 |
| Total CHD | 11 | 6 |
| Cor incomp (no CHD no VOC) | 4 | 0 |
| Periph arteriosclerosis | 5 | 3 |
| Hypertension (diast BP ≥100 mmHg) | 8 | 5 |

Table V *Cardiovascular status Women ≤65 years (see Table IV)*

| | Oophorectomised (*n*=17) | Controls (prolapses) (*n*=17) |
|---|---|---|
| Infarction | 1 | 0 |
| Angina pectoris | 4 | 0 |
| Pos working ECG no AP | 0 | 2 |
| Total CHD | 5 | 2 |
| Periph arteriosclerosis | 3 | 0 |

with prolapse (C) It was found that biliary symptoms (gallstone and/or inflammation of the gall bladder) was not much more common among women who had complete removal of the ovaries while cardiac symptoms and nervous complaints were significantly more common in this group Complete oophorectomy probably also leads to an increased frequency of fractures (almost significant) various other diseases and the need for medication

### *Special cardiovascular findings*

#### *Blood lipids*

The frequencies of various cardiovascular findings are given in Table IV

It is clear from the Table IV that no certain difference in frequency was found between the groups In patients below 65 years however the frequency of Coronary Heart Disease (CHD) and peripheral symptomatic arteriosclerosis tended to be higher in the oophorectomised group but the number of patients was small and the difference was not statistically significant (Table V)

The group of patients with signs of coronary disease included those who had no subjective symptoms suggesting CHD but in whom the working ECG suggested coronary insufficiency It is noteworthy that if those patients are not included in the CHD group which thus then consisted only of patients with frank myocardial infarction and angina pectoris the difference in the frequency of coronary disease in ages below 70 years was significant (Table VI)

No difference in blood pressure was found with certainty between the women after oophorectomy and the controls (Fig 1)

No change in the serum cholesterol or serum triglycerides could be demonstrated with certainty after oophorectomy in the series as a whole (Table VII)

In the lower ages i e below 60–65 years how

Table VI *Myocardial infarction (MI) and angina pectoris (AP) after castration*

| Age | | Oophorectomised | Controls | Diff |
|---|---|---|---|---|
| ≤60 | MI+AP | 2 | 0 | $p<0.05$ |
| | Miscellaneous | 3 | 9 | |
| ≤65 | MI+AP | 5 | 0 | $p<0.05$ |
| | Miscellaneous | 12 | 17 | |
| ≤70 | MI+AP | 9 | 1 | $p<0.05$ |
| | Miscellaneous | ?0 | ?? | |
| ≤75 | MI+AP | 11 | 3 | No significance |
| | Miscellaneous | 30 | ?6 | |

ever the cholesterol and triglyceride levels were higher in the women after oophorectomy than in the controls The difference was significant for serum cholesterol in ages below 60 and for serum triglycerides in the group below 65 years (Figs 2 and 3)

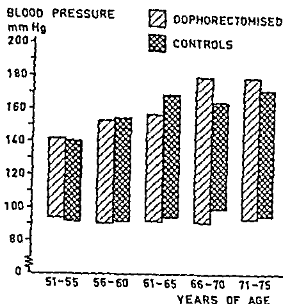


*Fig 1*

The serum lipid level in the oophorectomised patients with CHD did not differ from that in the controls with CHD Neither was any significant difference found in serum lipids between controls with and without demonstrable coronary disease But oophorectomised patients with CHD had a higher serum cholesterol level than those without CHD 283 and 249 mg/100 ml respectively ($p<0.05$)

When the postoperative group and the control group were pooled the cholesterol level was higher in patients with signs of CHD than in those without t no notable difference was found in serum glyceride concentration (Table VIII)

In contrast with what was found in the pooled material the serum triglyceride levels in patients with signs of peripheral vascular disease was higher than in patients without while no clear difference was found in the serum cholesterol level (Table IX)

*Osteoporosis*

The records showed an almost significant increase in the frequency of fractures in the women who had been castrated Detailed analysis of the case histories revealed that the frequency of fractures of

Table VII *Serum cholesterol and triglyceride levels*

| | Oophorectomised ($n=41$) | Controls ($n=?9$) | $P$ |
|---|---|---|---|
| Cholesterol mg/100 ml | 260 | 251 | n s |
| Triglycerides mmol | 1 ?4 | 1 09 | n s |

the radius was significantly increased (Table X) Alffram & Bauer (2) found a markedly increased frequency of radial fractures after the menopause in the women in Malmo In our group of women after oophorectomy this increase is pronounced Oophorectomy may thus accelerate osteoporosis a common predisposing cause of fractures 2 of the women had had 3 radial fractures and 1 woman had had 2 The 15 radial fractures reported had occurred in 10 women Fractures of the femoral neck showed the same tendency to increase in frequency as radial fractures while the frequency of vertebral compression and fractures showed no such tendency Thus body height did ot differ between post operative women—160 cm (150–171) and the controls—157 (146–168) Byt osteoporosis was significantly more pronounced in the oophorectomised women

Oophorectomy had thus increased the tendency to fractures osteoporosis and weakness of the bones

The cortex as measured by the method of Meema (36) was found to be thinner in the postoperative group (Table XI) The serum phosphorous but not the serum calcium was for some unknown reasons increased in the women after oophorectomy

Among the women after oophorectomy the skeleton was less brittle (fewer fractures) when the excretion of oestrogens was higher and that of ketosteroids lower and this was found both in women above and below 65 years

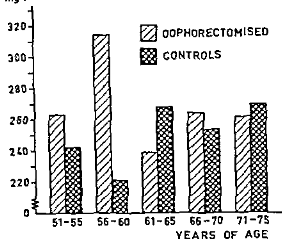


Fig 2

### *Hormonal balance*

Table XIII compares the excretion of hormones in the postoperative women and in the controls

The *significantly increased activity* of the adrenals after oophorectomy is noteworthy It was most pronounced in the younger group of women Also the oestrogens excreted had probably been derived from the adrenals It may therefore be a sign of adaptation and of the defence mechanism of the body initiated by the gonadectomy

Asymptomatic bacteriuria was found in 2 of the 32 sterilised women examined Cytological examination of smears revealed no signs of malignancy in 29 of the 32 women but atypia in 3 of them None of the smears showed signs of increased oestrogenic influence (acidophilia)

### *Psychiatric findings*

The psychiatric examination was limited to 20 of the 32 thoroughly investigated patients (Group A) summarised in Table XIV Only 3 (15%) were single and 3 were divorced None of these figures differ from those for the general population Seven women

Table VIII *Serum lipid levels in oophorectomised women+controls with and without signs of CHD*

| | CHD ($n=17$) | No CHD ($n=53$) | |
|---|---|---|---|
| Cholesterol | 280 mg/100 ml | 249 mg/100 ml | $p \leq 0\ 05$ |
| Triglycerides | 1 26 mmol | 1 15 mmol | n s |

Table IX *Serum lipid levels in oophorectomised women+controls with and without signs of stenosing peripheral arteriosclerosis*

| | P A ($n=8$) | No P A ($n=67$) | |
|---|---|---|---|
| Cholesterol | 266 mg/100 ml | 255 mg/100 ml | n s |
| Triglycerides | 1 50 mmol | 1 13 mmol | $p \leq 0\ 05$ |

were mothers at the time of the operation 4 of them had their children out of wedlock

In retrospect the most important result of the operation in the women s opinion was the sterility Twelve (60%) women said that the thought of the sterility was a terrible shock while 8 said it caused no problems or even a relief In the sample as a whole there was no relationship between childlessness and regret of sterility *Within the group of women who* had children before their operation however the 3 women with legitimate children regretted the sterility while the 4 with illegitimate children did not This latter distribution is hardly due to chance (exact $p=0\ 028$)

Judging from the brief review the psychological mechanisms used by the women for coping with the trauma of sterility varied Some of those who did not regret the condition evidently used denial and repression of their sorrow anger and grief Two women indicated that they accepted the sterility as a punishment for their illegitimate pregnancies

*Two women reported phobic reactions (fear of*

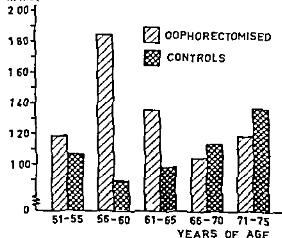


*Fig 3*

Table X *Fractures and osteoporosis*

| | Group | |
|---|---|---|
| | A+B | C+D |
| Total number of patients in group | 57 | 43 |
| Patients with fractures | 23 | 10 |
| Number and type of fractures | | |
| Radius | 15* | 2 |
| Femoral neck | 8 | 3 |
| Vertebral column | 2 | 1 |
| Others of known site | 5 | 1 |
| Others of unknown site | 2 | 5 |
| Total number of patients exam in regard of osteoporosis | 28 | 43 |
| Number of patients with Rtg osteoporosis | 6 * | 1 |

Table XI *Thickness of cortical bone of radius in mm (Meema) serum calcium and serum phosphorous in women without fractures in their history*

| Group | Corticalis thickness (mm) | Ca/s (mmol/l) | P/s (mg/100 ml) |
|---|---|---|---|
| A | 4 3 ($n$=13) | 4 71±0 18 ($n$=16) | 3 55* ±0 62 ($n$=17) |
| C | 4 6 ($n$=16) | 4 80±0 24 ($n$=26) | 3 05 ±0 63 ($n$=24) |

*being alone and fear of going out respectively)* after their operation One had fainting attacks for 10 years after the operation Epilepsy was suspected but not proved and several years later she reacted to domestic stress with clear-cut hysterical reaction She could not stand or walk One woman used projection she blamed her former fiancé in a very unrealistic way for having caused her illness None of the women blamed the doctors or the hospital To one woman the sterility was the tragedy of her life She was not informed about it until 3 years after the operation—it was a great shock she became depressed she gave up her fiancé and her career and moved to a small place until pension age At the investigation she was still very unhappy At least 6 of the women clearly described how they coped with the crisis they found a satisfactory substitute in work education creative activities or adopted children

At the examination 7 women presented symptoms and/or signs of nervous disorders The diagnoses were as follows

| *Pat No* | *Age* | *Diagnosis* |
|---|---|---|
| 1 | 64 | Anxiety neurosis |
| 3 | 68 | Subnormality |
| 4 | 56 | Depression |
| 5 | 69 | Subnormality+personality disorder |
| 7 | 59 | Character neurosis |
| 10 | 71 | Depression |
| 12 | 75 | Cerebral arteriosclerosis |

Patients 1 and 4 had had their nervous symptoms since the operation No 10 since she had been informed that she was infertile For the remaining 4 subjects there was no time relationship between the operation and the symptoms

The personalities and complaints of 5 women appeared normal for age 8 (40%) women were unusually vital active and sympathetic

The sexual activity of the women could be assessed in all cases except one in which the patient refused to discuss the matter (Table XV) One women had had no sexual relationship after the birth of her child 3 years before the operation Three women lost their libido after the operation The husband of one of them died the same year as her operation and she still mourned him In her second marriage she was frigid It is not possible to decide whether the operation or the bereavement was responsible for her frigidity Two more women became frigid later in life

Table XII *Fragility of skeleton and excretion of hormones*

| *Group A* | Oestrone (*µg/24 hours*) | Oestradiol (*µg/24 hours*) | 17 keto-steroids (*mg/24 hours*) | 17 OH keto steroids (*mg/24 hours*) |
|---|---|---|---|---|
| Castrated patients with fractures | | | | |
| ($n$=5) <65 years | 2 7 | 6 5 | 7 9 | 12 0 |
| ($n$=7) >65 years | 2 0 | 4 4 | 5 3 | 8 4 |
| Castrated patients without fractures | | | | |
| ($n$=10) <65 years | 3 0 | 6 1 | 6 6 | 10 8 |
| ($n$=6) >65 years | 3 1 | 9 6 | 3 7 | 8 1 |

Table XIII *Urinary excretion of total oestrogens and adrenocortical hormone metabolites*

| Group | | Low polar total oestrogens (µg/24 hours) | | | 17 keto steroids (mg/24 hours) | | | 17-OH keto steroids (mg/24 hours) | |
|---|---|---|---|---|---|---|---|---|---|
| Castrated (A) | | | | | | | | | |
| (n=15) <65 years | *p* | 11 2 | | *p* | 7 0 | *p* | *p* | 11 2 | *p* |
| (n=13) >65 years | | 8 9 | *p* | | 4 6 | | | 8 2 | |
| Controls (C) | | | | | | | | | |
| (n=17) <65 years | | 3 5 | | | 4 4 | | | 8 2 | |
| (n=15) >65 years | | 2 0 | | | 4 6 | | | 10 8 | |

one when she was informed of the infertility one after a hysterectomy 19 years after castration The sexual activity of the majority of the women thus seemed largely unaffected by the operation

With but one exception there was no relationship between the psychiatric variables and the results of the hormone analyses The exception was that in the women who appeared to be unusually mentally active the excretion of oestriol was *lower* (diff 1 867 *t*=2 508 *p*<0 25)

## DISCUSSION

Earlier comparisons of the incidence of CHD between oophorectomised women after oophorectomy and controls have given differing results In an autopsy series consisting of 49 women who had bilateral oophorectomy 2–42 years previously Wuest et al (64) found more coronary atheroma than in their control series But their series were small and the range of variation of the severity of the coronary changes was wide The difference in frequency between the series and the controls was largest in the subgroup that had undergone oophorectomy 5–9 years before death In another post mortem investigation Rivin and Dimitroff (52) reported a significantly higher frequency of pronounced atheroma of the coronary vessels in oophorectomised women than in the general population In a clinical investigation of such women compared with a personally selected control group consisting of women after hysterectomy as well as with an age-corrected group from the population of Framingham Robinson et al (19) found a significantly higher frequency of arteriosclerosis manifested clinically as CHD or peripheral vascular disease In a similar investigation Oliver & Boyd (43) found a higher concentration of cholesterol and coronary vascular disease more often after bilateral than after unilateral oophorectomy

In contrast with the above mentioned investigators however Novak & Williams (41) found no difference post mortem in the frequency of marked arteriosclerosis between women after oophorectomy and controls But their series included women who had had their operations only a few years before death as well as women who had reached such an age that the difference if any between the two groups must have been at least partly masked by senile arteriosclerosis If the women of advanced age be excluded the frequency of severe atheromatosis in the postopera

Table XIV *Group of women castrated early and examined psychiatrically*

| | |
|---|---|
| Age | |
| mean | 65 years |
| range | 54–85 years |
| *Civil status* | |
| unmarried | 3 15% |
| married before op | 6 |
| married total | 17 |
| divorced after op | 3 15% |
| Women with children | 7 |
| thereof illegitimate | 4 0% |
| Admits sterility being a trauma | 12 60% |

Table XV *Sexual activity of women castrated early*

| Before operation | After operation | | | |
|---|---|---|---|---|
| | Good | Poor | No exp | ? |
| Good excellent | 10 | 3 | – | – |
| Poor | – | 2 | – | – |
| No experience | 3 | – | – | – |
| Unknown | – | – | 1 | 1 |

ta Obst + 5)

31–75 865

tive group will be higher than in the controls In a well controlled investigation Ritterband et al (51) did not find the frequency of arterio sclerotic heart disease to be increased in women after oophorectomy But they reported a re markably low frequency of CHD about 9% in their group compared with usually about 20% in clinical studies by other investigators

The marked increase in the frequency of coronary vascular disease in women after 60 years of age occurs about 15 years after the natural menopause This increase in CHD is ascribed to the decrease in the synthesis of oestrogens at the onset of the menopause It was therefore considered legitimate to study the incidence of CHD in women whose ovaries had been removed at least 15 years previous ly On the other hand the increased occurrence of arteriosclerotic vascular changes in advanced age will probably mask any difference between normal and oophorectomised women regarding clinical signs of vascular disease of the heart and limbs if the interval is too long In our investigation this interval was on average 43 years This long interval proba bly masked the difference suspected in the total group in the frequency of CHD and in the concentra tion of the blood lipids apparent in the lower age groups

The difference observed in the serum cholesterol level between women with and without signs of CHD is well known Our finding that stenosing peripheral arteriosclerosis correlates better with the high triglyceride level than with the high cholesterol level is supported by earlier investigations (22 25 28 56) Neither in the Framingham investigation (19) of pa tients above 50 years was the cholesterol concentra tion found to be higher in patients with intermittent claudication than in those without

The investigations referred to above and observa tions made in the present study appear to warrant the conclusion that bilateral oophorectomy leads to a higher frequency of myocardial infarction and an gina pectoris Like Robinson et al (53) we found that if the criteria of the clinical diagnosis CHD are extended to include patients with a doubtful history or patients without symptoms but with specific ir regularities in electrocardiograms recorded during work it will diminish the difference between women with and without ovaries Asymptomatic persons with a working ECG suggesting coronary insuffi ciency however develop manifest coronary vascu lar disease more often than persons with a normal working ECG (5, 35 54) These observations might suggest that oophorectomy accelerates coronary stenosis and symptomatic coronary disease In fact in our series many of the younger women with such a disease in the postoperative group had already died This means that the group of survivors was selected in respect of CHD

The most comprehensive psychiatric review of castrated women that has hitherto been published is that by Pedersen (46) The present study differs from Pedersen s in two respects our series included only women whose operations were before the age of 30 and the interval between the operation and the re view was almost 4 times as long (means 41 5 years compared with 11 4 years)

During the interval between operation and follow up almost one third of the women in the original sample died From a psychiatric point of view this selection affects women with cerebral arteriosclerosis and those who committed suicide The effect of this bias on the result is uncertain The main subjective effects of the operation are menopause the hot flushes and the sterility In this review sterility was by far the most important effect and the one which to a large extent determined the total effect of the operation on the lives of the women To some women probably those whose lives had been less fortunate and whose social condi tions had been less satisfactory sterility meant a catastrophe or life long neurotic adaptation To other women it was a severe psycho traumatic crisis but one which they could cope with and they eventually found gratification in substitute activities In a broad psychological sense of the term oophorectomy does not differ from other kinds of psycho-traumatic crises

Pedersen refutes Bleuler s assumption of a general Endocrines Psychosyndrome in women after oophorectomy and proposes the term climacteric psychosyndrome for the vasomotor disturbances with hot flushes sweating tachycardia etc which are common features of both the normal and the artificial climacteric and due to oestrogen deficien cy

In the present sample the immediate mental reac tions to the operation were ascribed to the trauma of sterility This is high lighted by a woman who was normal until 3 years after the operation but when she was informed of the sterility she became depressed

Our impression from this small sample examined

is then that the nervous disorders following oophorectomy are of psychological rather than of endocrinological origin

It was noteworthy that judging from the histories and the personal interviews many of the women were unusually active energetic and sthenic This is in agreement with Pedersen s study where 41 % of the total sample was described as restless agile and hyperactive The most probable explanation of this observation is a psychological one i e women deprived of the possibility of giving birth direct their libidinal energy (in the psychodynamic sense of the word) to the outer reality On the other hand the finding in the present study that the group of women judged to be unusually active had a lower excretion of oestriol indicated that the high level of mental activity of these women was associated with the endocrinological effect of the castration This assumption is supported in Pedersen s study in which the sthenic traits were significantly more often observed in totally than in subtotally castrated women (49 and 15% resp $\chi^2=15\ 63$ 1 d f )

Pedersen s explanation that the sthenic traits were secondary to ANS (autonomic nervous system) symptom (hot flushes etc ) does not hold true for the present sample as most subjects had passed their period of hot flushes and no association could be found between duration of severity of these symptoms and the level of mental activity Three alternative hypotheses may perhaps explain the effect of castration on the mental activity

Ovarian gestagens (progesterone) are known to have a sedative effect on the CNS at least in large doses (37) It has been described as the passivity hormone preparing the women to the self-centred state of pregnancy (6) A life long deprivation of progesterone might allow for a higher level of mental activity

Low oestrogen level induces high levels of FSH which has a stimulatory influence on the CNS of the rabbit (55)

Castration increases the level of FSH–RF (gonadotropin releasing factor) in hypothalamus of rats (34 38) Whether this neurohormone has any influence on higher brain centres or on mental functioning is not known but recently a stimulating and mood-elevating effect of TSH RF (thyrotropin releasing factor) has been demonstrated both in normal (48 63) and in subjects with endogenous depression (29 47) If FSH RF has a similar effect or if castration has an effect on the synthesis and release of TSH RF the resulting increased mental activity might be explained through the hypothalamus

The absence of a gross effect of castration on sexual function finally confirms earlier observations

The increased vicarious adrenal activity in these women was noteworthy It has often been shown that the adrenal cortex produces steroid sex hormones and undergoes proliferative changes in gonadectomised animals (for ref see Thung (61)) Frantz and Kirschbaum (20) who studied gonadectomised mice observed that in some strains the pattern of the adrenocortical hormones was dominated by oestrogen in others by androgen

The adrenal cortex and its reaction to stress is of significance in coronary diseases It may also help to explain the osteoporosis and weakness of the bones As known rarefaction of bone occurs and gradually progresses in all women above 40 years of age This senile osteoporosis is thus more marked after the menopause Extensive decalcification with pathological osteoporosis cannot be explained by ageing alone It is of endocrine origin probably with involvement of the sex hormones Such hormones are produced not only by the ovaries but also by the adrenal cortex Primary or accompanying functional disorders of the adrenals may thus contribute to such osteoporosis Calcitonin might secondarily be involved in this mechanism (24)

The protective mechanism which women after oophorectomy have been deprived of is the monthly secretion of oestrogens–gestagens as well as the profound changes of pregnancy (prolonged haemodynamic adjustments etc ) This loss in such women on the other hand means elimination of factors probably playing a significant role in the causation of mammary tumours Remarkably enough none of these women had died from mammary carcinoma and of those alive only one of them had been operated on for such a condition Earlier experience has shown that women who have oophorectomy before the age of 40 develop cancer of the breast only one fourth as often as women in comparable groups (19 23)

Hormonal influence is no doubt operative in the initial phase of development of breast cancer Endocrine abnormalities—low androgen metabolite excretion—may be a primary factor long before

breast cancer develops (13) The extent to which prolactin is of importance in the pathogenesis and growth of breast cancer is at the moment a matter of debate Prolactin production probably is severely reduced during the life of women castrated when young Its blood level is now being studied in the group of women described and so are other gonadotropins and steroids Deep frozen blood and urine specimens are available for continued re search

In the light of our observations and earlier know ledge it would seem wise as suggested to substitute oestrogen in the artificial as well as in the natural menopause (21) Synthetic oestrogens may imply a certain risk of thrombosis Natural oestrogens seem to be preferable (66) especially since they also have a suppressive effect on triglyceri des and $\beta$-lipoproteins (32) Atrophic changes in the urogenital tract are counteracted by oestro gens which also have a beneficial well docu mented effect on ageing skin (50) Prophylactic treatment with oestrogen can prevent the develop ment of oesteoporosis (40) and should be useful in the prevention of psychic disorders owing to lack of oestrogen

It has been proposed that after the menopause and throughout the rest of life most women should re ceive substitution therapy with natural oestrogens and progesterone added to induce regular monthly bleeding (21) The control of the effect of such a treatment could then very well be included in regular periodic gynaecological health checks at which vag inal smears should be examined not only for malignant cells but also for the effect of oestrogen Such follow ups should of course also include de termination of the serum cholesterol and triglyceride levels hepatic enzymes E C G and determination of the bone density At these regular check ups close attention should be given to any occurrence of abnormal growths in the mammary glands too

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*Submitted for publication July 15 1974*

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Acta Obstet Gynecol Scand 54 463–467 1975

# TUBAL SURGERY

*Report of 101 Cases with Special Reference to the Experience of the Surgeon*

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*Abstract* A report is given of the results of tubal surgery in 101 cases selected from 851 sterility patients The follow up time was 3–10 years Ordinary surgical technique was applied with the use of prednisolone and antibiotics but largely without the use of polyethylene tubing or other splints Fourteen surgeons were involved Since the most experienced surgeon operated on about 30% of the cases an assessment of the effect of the surgeon s skill was possible The patients are divided into groups with regard to type of operation and the result is judged with regard to conception live birth ectopic pregnancy and abortion as well as with regard to patency After salpingolysis the conception rate was 52% and after salpingostomy 32% There was however a gap between the conception rate and the live birth rate especially for the salpingostomy cases The live birth rate was more than twice as high after salpingolysis than after salpingostomy The small lasting effect of cut adhesions is also demonstrated by the observation that combined unilateral or bilateral lysis did not interfere with the result after salpingostomy Contrary to this the patients conceived faster after salpingostomy than after salpingolysis There was no difference between the results in the patients operated on by the most experienced surgeon and in those treated by the thirteen less skilled gynecologists

The results of tubal surgery reported in the literature vary to a surprising degree This seems to depend on many factors e g the selection of cases the surgical technique the way in which the results are presented the length of follow up Much emphasis has been put on the experience of the surgeon (2 4) the use of high dosage of glucocorticoids (1 5) polyethylene or silastic hoods (3) and the application of an elaborate and meticulous technique (6) The size of the published series also varies considerably it takes time to accumulate a series of tubal surgical cases worth publishing Some workers have resorted to joint publishing (1)

The present report covers 7 years of tubal surgery in one clinic The patients were selected from an ordinary sterility clientele and operated on with an ordinary and somewhat variable technique Since many doctors participated in the treatment of the patients but the most experienced surgeon performed a sizeable proportion of the operations it was possible to obtain an assessment of the effect of the surgeon s experience

## MATERIAL

The 101 cases of tubal surgery reported here were selected from 851 patients who were investigated and treated because of sterility in our clinic during the years 1963–69 The selection was done mainly by means of hysterosalpingography using an aqueous contrast medium This investigation was performed in 74? patients and in 248 (33%) injuries of the oviducts were found Prior to surgery it was checked that other conditions for fertility were fulfilled by performing a seminal investigation of the husband a postcoital test and when a tuberculous salpingitis was suspected or the patient was oligomenorrhoeic an endometrial biopsy as well Usually the hysterosalpingography was supplemented with an insufflation with carbon dioxide Among the patients who underwent salpingolysis one-third had been subjected to a gynecological laparotomy and another third had been treated for an earlier salpingitis In the salpingostomy cases one fourth were aware of an earlier salpingitis The incidence of secondary sterility was close to 50% compared with 33% in the whole sterility group The time of follow up was 3–10 years

int

olysis Types of operation and re

| | Number of patients | Pregnant | Live birth |
|---|---|---|---|
| A *Unilateral* | | | |
| 1 Contralateral oviduct absent | 8 | 3 | 3 |
| 2 Contralateral oviduct normal | 4 | 1 | 0 |
| B *Bilateral* | 25 | 14 | 12 |
| Total | 37 | 18 | 15 |

Table III *Time between operation and conception Cumulative figures*

| | Salpingolysis | Salpingostomy |
|---|---|---|
| 6 months | 6 | 9 |
| 1 year | 7 | 15 |
| 2 years | 14 | 18 |
| 3 years | 15 | 19 |
| 4 years | 17 | 20 |
| 5 years | 18 | 21 |

## METHODS

*Surgical technique* The tubal reconstruction was performed by laparotomy except in one case where salpingolysis was done at laparoscopy The technique of *salpingostomy* was either a broader incision at the ampullar end with eversion of the two flaps or a small incision followed by blunt widening and in both cases placing of a few fine catgut sutures to keep the distal portion of the oviduct everted In 3 cases resection of hydrosalpinx was done Uterine suspension by shortening of the round ligaments was performed in a few cases No microsurgical technique was used Plastic tubular splints were used only at the uterotubal implantation operations

*Drug treatment* At the end of the operation a solution of 20 or 25 mg prednisolone 10 units hyaluronidase and 1 million i u penicillin G was instilled in the pelvis After salpingolysis and salpingostomy the same amounts were given by pertubal instillation combined with insufflation two or three times in the postoperative week and then repeated one or several times at monthly intervals Prophylactic antibiotics usually a combination of penicillin and sulphonamide were given routinely during the postoperative week

*Postoperative assessment of patency* This was done in all cases with insufflation usually several times 1–6 months after the operation In half of the cases hysterosalpingography was also performed at varying times after surgery

Table II *Results of salpingolysis*

| | Number | Per cent |
|---|---|---|
| Delivered of live baby | 15 | 41 |
| Perinatal death of baby | – | – |
| Ectopic pregnancy only | 2 | 5 |
| Abortion only | 1 | 3 |
| Pregnant | *18* | *49*[b] |
| Not pregnant | 19 | 51 |
| Total | *37* | *100* |

Correction for cases with one normal oviduct 15/33 = 45%
[b] Correction for cases with one normal oviduct 17/33 = 52%

*Surgeons* The most experienced tubal surgeon of the clinic performed 29 operations The rest were divided between 13 well trained but less experienced gynecologists (see Table VII)

## RESULTS

### *Salpingolysis*

The results of the different types of operation is given in Table I When bilateral salpinoglysis was performed more than one half of the patients became pregnant in unilateral cases where one oviduct was absent the result was less satisfactory Surprisingly none had a live birth in the 4 cases where one tube was normal

Table II gives the result for the patients with regard to the outcome of pregnancy When correction has been made for the cases with one normal oviduct the conception rate was 17 out of 33 (52%) and the live birth rate 15/33 (45%)

Table IV *Salpingostomy Types of operation and results*

| | Number of patients | Pregnant | Live birth |
|---|---|---|---|
| A *Unilateral* | | | |
| 1 Contralateral oviduct absent occluded or removed | 8 | 2 | 1 |
| 2 Lysis of contralateral oviduct | 12 | 6 | 4 |
| 3 Contralateral oviduct normal | 10 | 6 | 4 |
| B *Bilateral* | | | |
| 1 No lysis | 7 | 1 | 1 |
| 2 Unilateral lysis | 5 | 2 | 1 |
| 3 Bilateral lysis | 15 | 4 | 3 |
| Total | 57 | 21 | 14 |

Table V *Results of salpingostomy*

| | Number | Per cent |
|---|---|---|
| Delivered of live baby | 14 | 24 |
| Perinatal death of baby | 1 | 2 |
| Ectopic pregnancy only | 6 | 11 |
| Abortion only | – | – |
| Pregnant | *21* | *37*[b] |
| Not pregnant | 36 | 63 |
| Total | *57* | *100* |

Correction for cases with one normal oviduct 10/47 = 21%
[b] Correction for cases with one normal oviduct 15/47 = 32%

The total number of pregnancies recorded in this group was 22 There were only three ectopic pregnancies and two abortions The patency rate was 35 out of 37 (95%) The time interval between operation and conception is recorded in Table III

The results were the same for patients operated on by the most experienced surgeon and patients treated by the other doctors see Table VII

*Salpingostomy*

Table IV shows that the results did not differ in the unilateral cases when the contralateral tube was normal and when a salpingolysis was performed on the contralateral tube When the contralateral tube was absent or occluded the result was worse and equalled the result in the bilateral cases It is clear from the bilateral cases that a combined lysis did not influence the result significantly No pregnancy occurred in the 3 cases where a resection of the ampullar end was performed

Table V gives the results for the whole group with regard to resulting pregnancy After correction for the cases with one normal oviduct the conception rate was 32% and the live birth rate 21% These figures indicate a high frequency of pregnancy failure in this group Actually among 32 pregnancies recorded there were 7 ectopic pregnancies (23%) 5 abortions (15%) and one perinatal death

The patency rate was 46 out of 57 (81%) The time lapse between operation and conception is given in Table III In this group also the results were very similar for the most experienced surgeon and the others (Table VII)

*Uterotubal implantation*

The types of operation and the results for this small group of 7 patients where a salpingitis isthmica nodosa was found in 5 cases is given in Table VI The single live birth in this group cannot be ascribed to the operation since it occurred in a case where the contralateral tube was normal In the other case where a conception followed the contralateral tube was also normal but the implanted tube was the site of the ectopic pregnancy In the three bilateral cases which were all combined with salpingostomy the postoperative hysterosalpingography showed bilateral occlusion of the uterine end of the tube in 2 cases and of the fimbrial end in one case

Table VI *Uterotubal implantation Types of operation and results*

| | Number of patients | Pregnant | Live birth |
|---|---|---|---|
| A *Unilateral* | | | |
| 1 Contralateral oviduct normal | 3 | 2 | 1 |
| 2 Bilateral salpingolysis and salpingostomy | 1 | – | – |
| B *Bilateral* | | | |
| 1 Contralateral salpingostomy | 1 | – | – |
| 2 Bilateral salpingostomy | 1 | – | – |
| 3 Bilateral salpingolysis and salpingostomy | 1 | – | – |
| Total | 7 | 2 | 1 |

## DISCUSSION

The conception rate after salpingolysis varies between 25 and 60% It is usually lower after salpingostomy averaging around 20% and varying between 5 and 50% For implantation operations which are less often performed the figures vary still more and are usually even lower The conception rate of the present series 52% after salpingolysis and 32% after salpingostomy seems to reach a good international standard This is rather remarkable because the patient material represented an everyday sterility clientele and the selection for surgery was done without intention of publicity

*with regard to the experience of*

| | No of cases | Pregnant *n* | % | Live birth *n* | % |
|---|---|---|---|---|---|
| A *Salpingolysis* | | | | | |
| Most experienced surgeon | 8 | 4 | 50 | 4 | 50 |
| 8 others | 29 | 14 | 48 | 11 | 38 |
| B *Salpingostomy* | | | | | |
| Most experienced surgeon | 17 | 6 | 35 | 4 | 24 |
| 12 others | 40 | 15 | 38 | 10 | 25 |
| C *Uterotubal implantation* | | | | | |
| Most experienced surgeon | 4 | 1 | | 1 | |
| 3 others | 3 | 1 | | – | |
| D *Total material* | | | | | |
| Most experienced surgeon | 29 | 11 | 38 | 9 | 31 |
| 13 others | 72 | 30 | 42 | 21 | 29 |

Moreover many and some less experienced gynecologists were involved and the surgical technique was hardly sophisticated

A notable finding in this study was however the equal results achieved by the group of 13 less experienced doctors and the most skilled surgeon Knowledge of the basic principles of tubal surgery is indispensable for a good result but experience is probably more decisive for the result with regard to a proper selection of cases than with regard to surgical performance This is also indicated by the failures of uterotubal implantation though the few cases of this type of reconstruction do not permit any further conclusions

Table VIII *Total series of tubal surgery Results*

| | Number of patients | Per cent |
|---|---|---|
| Delivered of live baby | 30 | 30 |
| Perinatal death of baby | 1 | 1 |
| Ectopic pregnancy only | 9 | 9 |
| Abortion only | 1 | 1 |
| Pregnant | *41* | *41*[a] |
| Not pregnant | 60 | 59 |
| Total | *101* | *100* |

Correction for cases with one normal oviduct 25/84=30%

[a] Correction for cases with one normal oviduct 29/84=35%

The gap between conception rate and live birth rate after tubal surgery has been demonstrated by others and is apparent in this report especially for the salpingostomy cases The live birth rate was more than twice as high after salpingolysis than after salpingostomy The high frequency of ectopic pregnancy after salpingostomy shows that the formation of a hydrosalpinx means a more extensive injury to the oviduct than mere adhesions The unnoticeable effect of a combined lysis on the result of salpingostomy also shows that adhesions after cutting have very little influence on the function of the oviduct The observation that the patients became pregnant faster after salpingostomy than after salpingolysis seems incompatible with these findings The difference between the number of patients who conceived within a year after surgery is however statistically significant ($p<0.05$) The explanation for this might be that salpingostomy means the removal of a cause of absolute sterility while adhesions usually represent a relative sterility factor

The results in the total series of tubal surgery presented in this report are shown in Table VIII The conception rate was 35% and the live birth rate 30% (after correction for cases with one normal oviduct) For the whole sterility clientele the conception rate was 45 4% and the live birth rate 38 7% When it is realized that more than 30% of the sterility clientele had injured oviducts and that most other sterility factors had been ruled out before surgery a comparison of these figures demonstrates that tubal injury represents a serious threat to fertility—a threat which is all too seldom overcome

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*Submitted for publication August 4 1974*

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Acta Obstet Gynecol Scand 54 469–477 1975

# A TECHNIQUE FOR MONITORING ENDOMETRIAL OR DECIDUAL BLOOD FLOW WITH AN INTRA UTERINE THERMISTOR PROBE

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*Abstract* A technique is described for studying en dometrial or decidual blood flow by introducing a thermistor mounted in a flexible catheter into the uterus The technique was first tested in model experiments and in the pregnant rabbit and was then used to study the blood flow of the human uterus In pregnant women the thermistor was introduced between the decidua and fetal membranes and in non pregnant women it was applied to the endometrium of the fundus Intra uterine pressure was recorded simultaneously No complications were en countered in either pregnant or non pregnant patients Decidual or endometrial blood flow remained steady over long periods There were transient fluctuations about the mean level usually associated with myometrial contrac tions but these could easily be distinguished from changes in the level of blood flow evoked by administration of vasoactive drugs

At any stage of pregnancy a pronounced decrease in maternal placental blood flow will place the fetus at risk There is thus an obvious need for a simple and reliable method to enable the clinical evaluation of drugs that may be given in the course of pregnan cy such as the sympathomimetics introduced for the inhibition of premature labour A similar tech nique is required for the study of uterine blood flow in dysmenorrhea and to evaluate the action of drugs including contraceptive agents upon the blood flow of the non pregnant human uterus

The aim of the present study was to test the suitability for these purposes of a thermistor flowmeter which enables relative values for local tissue blood flow to be obtained by a virtually non traumatic technique The use of thermistors in uterine blood flow studies was first advocated by Brotanek Kazda and Roth in 1962 (2) It is remark able that since then thermistor flowmeters have not found a more general application in obstetrics and gynaecology After having used and studied the Brotanek technique we found that it must be mod ified in several important respects to enable routine studies of the uterine circulation We shall therefore give a detailed account of our present technique and an evaluation of its usefulness and reliability made in a model system in the pregnant rabbit uterus and in the pregnant or non pregnant human uterus

## MATERIAL AND METHODS

### *Principle*

The resistance of a thermistor alters greatly with small variations in temperature These alterations can be meas ured by including it in one arm of a Wheatstone bridge The thermistor itself can be made very small and mounted in the tip of an hypodermic needle or catheter probe for insertion into tissues or body cavities being connected to the rest of the bridge by a cable Provided that the current through the thermistor has a low heating effect the thermistor will assume the temperature of the surrounding tissue and its resistance provides a measure of this temp erature (thermometric principle) If the effect is increased heating the thermistor to slightly above tissue tempera ture heat will be lost to the surroundings Given that the current is kept constant and tissue temperature remains unchanged the temperature assumed by the thermistor and thus its resistance will reflect the rate of heat loss (anemometric principle) This will vary with the tissue blood flow and a thermistor operating anemometrically can therefore be used to monitor changes in the rate of blood flow

### *Apparatus*

The thermistor probes chosen were of the catheter type YSI 511 and YSI 5 0 with time constants of 0 2 sec and

0 1 sec respectively (Yellow Springs Instrument Co Inc Yellow Springs Ohio) The YSI 520 which was mounted in teflon tubing (o d 1 0 mm) proved to be more robust than the YSI 511 mounted in polyethylene tubing (o d 0 6 mm) A few trials were made with a thermistor mounted in an hypodermic needle as described by Brotanek et al (7)

The arrangement of the Wheatstone bridge is shown in the circuit diagram (Fig 1) When the thermistor was operated anemometrically to monitor blood flow a relatively high effect was needed This was obtained from an ordinary 45 V battery stabilized with the aid of an integrated circuit (μA 723) to prevent a constant drift in output voltage due to discharge of the battery When the thermistor was operated thermometrically to measure tissue temperature a low effect was required In this situation we used a 1 35 V mercury cell which supplied only 50 μW to the thermistor and did not require stabilization The instrument was calibrated for temperature measurement by recording the output voltage when the thermistor was substituted by fixed resistances chosen with reference to the temperature resistance curve of the thermistor

The thermistors employed had a negative temperature coefficient i e their resistance decreased with increasing temperature and vice versa An increase in tissue blood flow causing greater heat loss from the thermistor was thus reflected as an increase in thermistor resistance Changes in resistance resulting in an imbalance of the Wheatstone bridge were recorded on a potentiometer writer (Rika Denki Tokyo)

A detailed temperature resistance curve was constructed for the YSI thermistors between 30°C and 46°C using a thermostatically controlled water bath and a digital voltage resistance meter (DANA model 350 DANA Laboratories California) With the aid of this curve it was possible to determine the temperature assumed by the thermistor under various experimental conditions since the resistance could be obtained by measuring the voltage and current across the thermistor and applying Ohm s law Plug in connections to the two thermistor leads were established for this purpose The measurements were made with an AVO meter (Simpson model 260 Simpson Electric Co Chicago Illinois)

### *Model experiments*

The following experiments were performed to establish the influence upon the thermistors of variations in environmental temperature and flow velocity A length of polyethylene tubing (o d /i d =5 5 mm/ 4 0 mm) was immersed in a thermostatically controlled water bath It had one end open to the bath and the other attached to a piece of rubber tubing that led through a peristaltic pump (Watson Marlow Ltd England) which maintained a constant flow of bath water through the tubing The thermistor probe was threaded into the tubing and the output voltage from the Wheatstone bridge measured by a digital voltage resistance meter (DANA model 350) Values were obtained for flow velocities between 4 8 and 131 0 cm/min as well as zero flow at bath temperatures of 37°C 38°C and 39°C The temperatures assumed by the

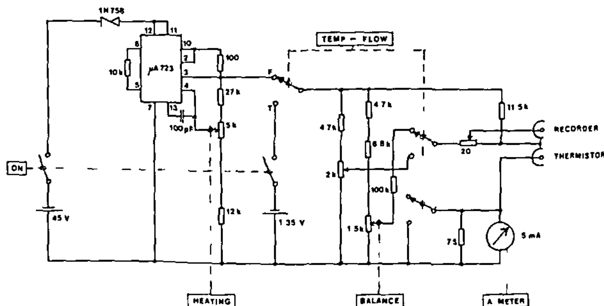



*Fig 1* Circuit diagram (resistances in ohms) The controls are few and simple to operate When the apparatus has been switched on and flow recording selected the heating current is adjusted to ca 4 7 mA and the Wheatstone bridge is balanced by a variable resistance to give a suitable deflection on the recorder When temperature recording is selected no adjustment of the heating current is necessary but the instrument must be calibrated as described in the text

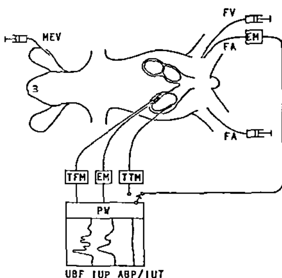



*Fig 2* Schematic drawing of experimental arrangement in the rabbit General anaesthesia is given in a marginal ear vein (*MEV*) Local blood flow and temperature are measured by a thermistor flowmeter (*TFM*) and thermistor temperature meter (*TTM*) respectively with probes in the right horn of the uterus Intra uterine pressure is transmitted through a sponge tipped catheter and measured by an electromanometer (*EM*) Drugs are given through catheters inserted in a femoral vein (*FV*) or in the right femoral artery (*FA*) Arterial blood pressure is recorded electromanometrically from the left femoral artery Uterine blood flow (*UBF*) intra uterine pressure (*IUP*) and arterial blood pressure (*ABP*) or intra uterine temperature (*IUT*) are recorded on the potentiometer writer (*PW*)

thermistors were also determined as described above The heating effect was kept constant throughout these experiments and was similar to that used in the subsequent trials

### *Studies in the rabbit uterus*

Pregnant rabbits were chosen to test the apparatus *in vivo* They were anaesthetized with intravenous pentobarbitone sodium (mebumalnatrium 6% ACO Sweden) the level of anaesthesia being kept as constant as possible throughout each experiment The experimental arrangement is shown in Fig 2 Variations in uterine blood flow were monitored by a thermistor probe inserted in the lumen of the right uterine horn through a small incision at the tubal end When intra-uterine temperature measurements were desired we used a second thermistor probe operated thermometrically A sponge tipped vinyl catheter (1) was introduced through the same incision to enable electromanometric registration of intra-uterine pressure in the immediate vicinity of the thermistor probe The tips of the probes and of the catheter were placed between the fetal membranes and the uterine wall and were thus closely apposed to the decidua The abdomen was closed but by thrusting the bulb of a thermometer between two sutures we were able to keep a continual check upon intra abdominal temperature This was kept as steady as possible with the aid of an electric blanket under the rabbit

### *Studies in the human uterus*

The instrument was tested clinically in patients whom had been informed of the nature of the trial and had consented to participate

The first recordings from the pregnant human uterus were made with the thermistor mounted in an hypodermic needle that was inserted in the uterine cervix as described by Brotanek et al (2) This method was used in six women admitted for therapeutic abortion in the 9th to 12th week of pregnancy The method finally adopted for monitoring uterine blood flow in the pregnant uterus was however to insert an YSI 511 or YSI 520 thermistor probe through the cervical canal and locate its tip between the parietal decidua and the fetal membranes (Fig 3) Intra-uterine pressure in the vicinity of the probe was recorded simultaneously through a saline filled sponge tipped catheter fastened to the probe by a silk ligature (Fig 4) Recordings were obtained from patients undergoing therapeutic abor

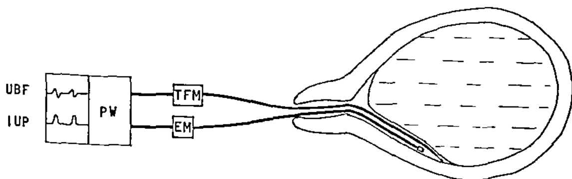



*Fig 3* Schematic drawing of experimental arrangement for intra-uterine recording in pregnant women Local uterine blood flow is monitored by a thermistor flow meter (*TFM*) and intra-uterine pressure by an electromanometer (*EM*) The thermistor probe and sponge tipped catheter are placed between the decidua and the fetal membranes Uterine blood flow (*UBF*) and intra uterine pressure (*IUP*) are recorded on the potentiometer writer (*PW*)

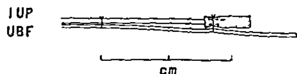



*Fig 4* Sponge tipped catheter for registration of intra uterine pressure (*IUP*) and thermistor probe for recording of uterine blood flow (*UBF*) as placed between the fetal membranes and the decidua

tion by the extra amniotic instillation of hypertonic saline solution (4) The thermistor probe and catheter were introduced concomitantly with the instillation of saline solution or 24 to 48 hours thereafter

In non pregnant women endometrial blood flow was recorded with an YSI 5 0 thermistor probe and intra uterine pressure with a Micro tip pressure transducer (Model PC 470 Miller Instruments Houston Texas) The thermistor and transducer catheters were fastened together by silk ligatures so that the thermistor protruded about three millimeters beyond the transducer tip After measuring the depth of the cervico-uterine canal with a sound the receptors were so inserted that the thermistor came to be pressed firmly against the endometrium of the fundus It was held in this position by the rigidity of the transducer catheter In order to support the catheters and prevent them from slipping out of place they were surrounded in the vagina by sterile compresses The procedure described was essential to prevent recording artefacts due to displacement of the thermistor from the endometrium during uterine contractions Insertion of the receptors was facilitated by first placing a sterile plastic tube (i d 4 mm) in the cervical canal The catheters were threaded through the tube which was then withdrawn

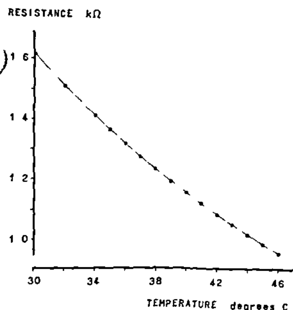



*Fig 5* Relationship between temperature and resistance for thermistor YSI 511 The relationship for thermistor YSI 520 was almost identical

## RESULTS

### *Model experiments*

The relationship between thermistor resistance and thermistor temperature was almost identical for the YSI 511 and YSI 520 probes between 30°C and 46°C (Fig 5) Knowing this relationship it could be established that the current used to heat the thermistor in flow studies maintained it at a temperature of 1 5°C to 4 5 C above an environmental temperature of 42°C to 37°C The values presented in Table I are taken from model experiments but comparison of the thermistor temperature in utero with the intra uterine temperature gave identical results The Table does not reflect the variations in thermistor temperature caused by increased cooling at highe flow velocities as these were of a smaller magnitude than the changes caused by one degree shifts in bath temperature The effect upon thermistor temperature of varying the flow velocity is however illustrated in Fig 6 which shows how the output voltage from the flowmeter varied with the flow velocity at three different bath temperatures

### *Studies in the rabbit uterus*

It is evident from Fig 6 that alterations in the output voltage from the flowmeter will reflect fluctuations in the temperature as well as in the blood flow of the decidua This posed more of a

Table I *Temperature assumed by the YSI 520 thermistor at various environmental temperatures C*

The thermistor was immersed in a thermostatically controlled water bath and operated anemometrically with the same heating effect as in subsequent in vivo trials

| Temperature of bath | Temperature assumed by thermistor | Temperature difference thermistor-bath |
|---|---|---|
| 37 | 41–41 5 | 4 0–4 5 |
| 38 | 4? | 4 0 |
| 39 | 43 | 4 0 |
| 40 | 43 5 | 3 5 |
| 42 | 43 5–44 | 1 5–2 |

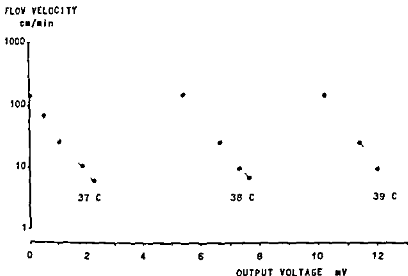



*Fig 6* Variation in output voltage from the flowmeter with flow velocity and bath temperature

problem in the animal experiments than in the clinical studies as the ability of the rabbit to regulate body temperature is adversely affected by barbiturate anaesthesia Furthermore since the probe was inserted in the uterine horn at laparotomy some cooling of the uterus was unavoidable As a result there was a stabilization period of 5 to 10 min following closure of the abdomen during which a gradual return of intra uterine temperature to its normal level caused a marked increase in the output voltage from the flowmeter under constant temperature conditions this would be indicative of a strong decrease in blood flow A similar effect could be achieved by injecting a small volume of cold saline into the uterus through the sponge tipped catheter The use of this type of catheter to record intra uterine pressure did not otherwise affect our results as could be confirmed by substituting it in one experiment by a closed catheter of the balloon type

In general decidual blood flow remained fairly steady over long periods but there were spontaneous fluctuations about the mean level most of which were associated with myometrial contractions If the contraction was a moderate one there was generally a coincident increase in flow (Fig 7 contraction B) Stronger contractions caused more complex fluctuations the increase in flow was interrupted as the contraction increased in intensity resulting in a dip corresponding to the intra uterine pressure maximum but there was a second increase in flow as the pressure decreased again (Fig 7 contraction A) These transient fluctuations in decidual blood flow could readily be distinguished from the changes elicited by intravenous infusion of vaso-active drugs which followed expected patterns

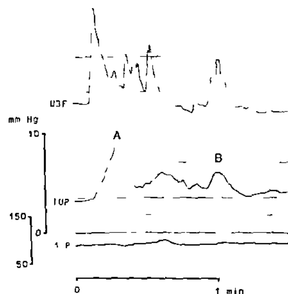



*Fig 7* Variations in local decidual blood flow of the pregnant rabbit uterus with changes in intra uterine pressure The smaller contraction (*B*) is accompanied by an increase in blood flow At the larger contraction (*A*) the initial increase in blood flow is followed by a dip that coincides with the intra uterine pressure maximum

3 –75 865

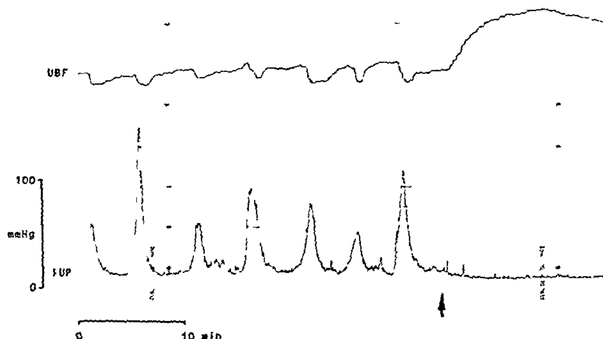



*Fig 8* Recording of local endometrial blood flow (*UBF*) and intra uterine pressure (*IUP*) from a healthy woman three days before the onset of menstruation The receptors were introduced through the cervix and placed between the endometrial walls near the fundus of the uterus Spontaneous contractions of high amplitude and long duration occur at regular intervals and a transient decrease in blood flow accompanies each contraction Following the intravenous injection (indicated by the arrow) of 500 µg terbutaline (Bricanyl® Draco Sweden) the uterus ceases to contract and there is a well-defined increase in endometrial blood flow

### *Studies in the human uterus*

Recording from a thermistor needle in the cervix of the pregnant uterus was abandoned due to technical difficulties including local haemorrhage after withdrawal of the needle Introduction of a thermistor probe and sponge tipped catheter between the decidua and fetal membranes was however easily performed and caused no great discomfort to the patient No complications such as accidental rupture of the membranes or damage to the fetus were seen Once the receptors were in place monitoring of the local blood flow could normally be continued even after spontaneous rupture of the membranes indicating that the thermistor was still closely apposed to the decidual wall It was not however possible to obtain a reliable recording if the probe was inserted after rupture of the membranes had already occurred

Introduction of the thermistor and transducer probes into the non pregnant uterus was also achieved without undue discomfort to the patient and satisfactory records of the endometrial blood flow could be obtained There were no complications such as infection of the genital tract or bleeding after removal of the probes

In general local uterine blood flow remained steady over long periods The most notable fluctuations occurred when the uterus contracted Thus there were transient decreases in blood flow concomitant with strong co-ordinated contractions of long duration (Fig 8) During unco-ordinated contractions of lesser duration however we often observed brief increases in blood flow similar to those seen in the rabbit experiments In recordings from both pregnant and non pregnant patients the effect of vasoactive drugs upon uterine blood flow could readily be distinguished from the fluctuations occasioned by uterine contractions there being a clear alteration in the mean blood flow during vasoconstriction (Fig 9) or vasodilatation (Figs 8 and 10)

UBF

200

mm Hg IUP

0

0 10 ml

ig 9 Recording of local endometrial blood flow (*UBF*) nd intra uterine pressure (*IUP*) from a healthy woman 56 iours before the onset of menstruation The receptors vere placed between the endometrial walls of the uterus Following the intranasal administration (indicated by arrow) of 5 mg *N* α triglycyl lysine vasopressin (Glypressin® Ferring Sweden) there is a marked decrease in endometrial blood flow a rise in the basal tonus of the uterus and a change in the pattern of uterine contractions

## DISCUSSION

The resistance of the thermistors employed in this study exhibits a practically linear relationship to temperature within the relevant temperature range (Fig 5) It must however be borne in mind that thermistor temperature and resistance are dependent upon three main factors The first of these is the heating effect supplied to the thermistor which should be kept constant It seems imperative to stress this point since thermistor flowmeters with an unstabilized power supply are commercially

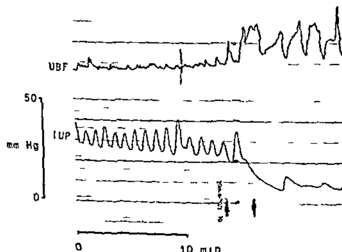



*Fig 10* Recording of local decidual blood flow (*UBF*) and intrauterine pressure (*IUP*) from a woman in the 16th week of gestation 48 hours after extra amniotic injection of 140 ml 20% saline solution The receptors were placed together between the fetal membranes and the decidua A continuous intravenous infusion of oxytocin 0 12 IU/min was given At the intravenous injection of 250 μg terbutaline (indicated by arrows) uterine tonus decreases and both the amplitude and frequency of contractions diminish The pattern of local blood flow changes markedly there is an increase in the mean level and the fluctuations are of greater amplitude and lower frequency

available Secondly the temperature and resistance of the thermistor is strongly influenced by alterations in environmental temperature even when it is operated anemometrically (Fig 6) This caused some inconvenience in the animal experiments as already mentioned but is probably less important in a clinical context

The rate of heat loss from the thermistor which is the third factor affecting its temperature and resistance will reflect the magnitude of blood flow in its immediate vicinity The flowmeter cannot at present be calibrated to translate heat loss to volume flow but the variations in output voltage will nevertheless supply important information about the direction of changes in blood flow and a relative indication of their magnitude This is an important advance since most of the methods that have been devised to measure uterine blood flow and its distribution in experimental animals are not clinically applicable In women evaluation of uterine blood flow has been attempted primarily by isotope clearance techniques employing $^{24}$Na (7) or $^{133}$Xe (8) These do not allow continuous recording of blood flow over long periods of time and moreover require expensive and cumbersome equipment

It must be stressed that the thermistor registers only changes in local tissue blood flow Since blood flow may vary independently both within and between the various tissues of the uterus the location of the thermistor becomes of great importance for correct interpretation of the results We can see no obvious advantage in measuring the blood flow in the isthmus or cervix (2 9 10) In the pregnant uterus the cervix is remote from the crucial region the site of attachment of the placenta and it is not subject to the pressure increases in the uterine lumen occasioned by myometrial contractions This is one pertinent reason for electing to place the thermistor probe in the uterine lumen between the fetal membranes and the decidua Another is that introduction of the thermistor probe can readily be combined with the insertion of a receptor for the recording of intra uterine pressure

In studies of the non pregnant human uterus it is often most relevant to record the endometrial component of uterine blood flow and once again convenient to insert the receptors for blood flow and intra uterine pressure together It should be noted that the temperature assumed by the thermistor in the uterus was only 4°C above the tissue temperature This temperature difference is not in itself sufficient to influence the local muscle blood flow (5) Our technique thus fulfils the obvious requirement of not altering the variable to be measured

The brief decreases in local uterine blood flow seen during strong co-ordinated uterine contractions can be attributed to the compression of blood vessels by the contracting muscle In some of our patients however myometrial contractions were associated with transient increases in local blood flow Prill (9) has reported a similar effect of periodic uterine contractions upon myometrial blood flow in the isthmic region A plausible explanation of this finding is suggested by the results obtained in animal experiments In the pregnant rabbit contraction waves are propagated peristaltically along the uterine horn (3) It is thus a reasonable assumption that there will be a redistribution of blood flow from contracting to non-contracting areas that is from areas of high resistance to areas of low resistance to blood flow This would entail an increase in local blood flow as the contraction wave approached the site of the thermistor a decrease in flow as the contraction reached this site and a further increase as the wave passed further along the uterine horn This sequence of events was in fact commonly observed In the human uterus where both local and propagated contractions are known to occur (6) the sequence of local blood flow changes during many contractions was similar to that observed in the rabbit suggesting a redistribution of blood flow from contracting to non contracting areas

The essential point however is that the effects of vasoactive drugs upon local uterine blood flow can readily be distinguished from fluctuations due to uterine contractions A thermistor flowmeter with the modifications described in this paper can thus be recommended for use in the assessment of the acute effects of drug administration and other procedures upon endometrial or decidual blood flow

## ACKNOWLEDGEMENTS

We wish to thank Mr Lars-Goran Angantyr for his invaluable technical advice This investigation was supported by a grant from the Ford Foundation

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*Submitted for publication Aug 13 1974*

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Acta Obstet Gynecol Scand 54 479–484 1975

# METHODS OF CALCULATING UTERINE BLOOD FLOW FROM THE WASH OUT CURVES OF INTRA ARTERIAL AND LOCAL INJECTIONS OF ¹³³XENON

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*Abstract* The wash-out curve from the human non pregnant uterus of intra arterially and locally injected ¹³³Xenon can be broken down into two sometimes three components It has not been shown that these components are physiologically meaningful However if it is assumed that the uterus is composed of two or three compartments perfused in parallel it is possible to calculate total blood flow per unit weight using relative weights of the compartments and compartment blood flows Relative weights may be determined from the amount of isotope present in each compartment at the start of the wash-out The possibly false assumption of a certain number of compartments has in this study been avoided by evaluating curves after intra arterial injections according to the stochastic method of Zierler and a modified Zierler analysis disregarding any elimination of isotope left after 20 minutes After local injections initial slope analysis has been used The equation for values according to Zierler as a function of compartment values is $y=0\ 9772x-0\ 5921$ for modified Zierler analysis values $y=1\ 2582x-2\ 3993$ and for initial slope values $y=0\ 8679x-1\ 3412$ The conclusion is that compartment analysis may be a good alternative for calculation of mean blood flow even if it has not been shown that individual compartment values are physiologically meaningful

In a previous report (3) the elimination curves from the human non pregnant uterus of intra arterial and local injections of ¹³³Xenon were described It was concluded that the elimination curve is as a rule a multi-exponential function which may be broken down into two sometimes three components with clearly different half times However no attempt was made to interpret the curves in terms of uterine blood flow or regional blood flow Previous authors have all used the so called initial slope method when calculating uterine blood flow from isotope wash out curves (2 6 7 8 10) As will be shown below this method is acceptable only under certain conditions Different ways of calculating total blood flow within the isotopelabelled region will be discussed

*Theoretical considerations*

The equations describing isotope elimination in mono- and multi-compartment tissues have been described previously The conditions for calculation of blood flow in individual compartments and equations for this were also given (3) However equations for calculation of total blood flow in multi-compartment tissues were not described If the percentage weights of compartments are known it is possible to calculate blood flow per unit weight of the composed tissue according to eq 1

$$F_{tot}=\sum_{1}^{n}\frac{W_i\ F_i}{100} \tag{1}$$

$F_{tot}$=blood flow per unit weight of composed tissue $W_i$=percentage weight of individual compartments and $F_i$=blood flow per unit weight of individual compartments

In the special situation where the concentration of isotope is the same in all compartments the amount of isotope in each compartment is a measure of the weight of the compartment Such a situation follows a local injection of isotope or a prolonged intra arterial infusion provided the partition coefficient is the same in all compartments

Eq 1 may be modified by inserting percentage activity instead of percentage weight provided activity in all compartments is recorded with equal efficiency (eq 2)

$$F_{tot}=\sum_{1}\frac{A_i\ F_i}{100} \tag{2}$$

$A_{0i}$=percentage activity in each compartment at the end of labelling

In this special situation however the initial slope of the semilog elimination curve represents the mean tissue blood flow as shown by Ingvar & Lassen (5)

By modifying eq 2 it is possible to calculate blood flow omitting for instance the region with the highest or the lowest perfusion rate The use of eq 2 or the initial slope method is limited to the situation of identical saturation of all compartments After a bolus injection into the artery supplying a number of in parallel compartments each compartment is labelled in proportion to its blood flow The amount of isotope brought to each compartment is then a function of blood flow per unit weight and actual weight of that compartment (eq 3)

$$A_0 = f[FW] \tag{3}$$

$A_0$=amount of isotope brought to the compartment

If this equation is solved for tissue weight we have a possibility of determining the percentage weight of any of several compartments in a composed tissue according to eq 4

$$W_i = \frac{\frac{A_{0i}}{F_i}\ 100}{\sum_1 \frac{A_{0i}}{F_i}} \tag{4}$$

$W_i$=percentage weight of individual compartment ~percentage activity in individual compartment the end of labelling and $F_i$=blood flow per unit ight of individual compartment

The total blood flow is also calculated by inserting the percentage weight values into eq 1 Hereby we get eq 5 cf (1 4)

$$F_{tot} = \frac{100}{\sum_1 \frac{A_{0i}}{F_i}} \tag{5}$$

A different type of analysis is proposed by Zierler (11) His type of analysis does not require homogeneous tissue or a certain number of homogeneous compartments nor is diffusion equilibrium between tissue and blood required The calculation of blood flow is made according to eq 6

$$F = \frac{H_{max}}{A}\ S\ c\ \ 100\ ml\ \ min^{-1}\ \ 100\ g^{-1} \tag{6}$$

$H_m$ =maximum height of the externally recorded activity curve $A$=area under the curve and $S$=partition coefficient in ml blood per g tissue

This type of calculation can be made after local or intraarterial injections of isotope when the injection is made over a very short period of time

A modification of this equation is often used for the calculation of cerebral blood flow Here the elimination curve is not followed down to background level All activity after a certain time is disregarded The modified equation then is eq 7 (4)

$$F = \frac{H_{max} - H_t}{A_{corr}}\ S\ \ 100\ ml\ \ min^{-1}\ \ 100\ g^{-1} \tag{7}$$

The differences between the two types of calculations are illustrated by Fig 1

## METHODS AND MATERIAL

Injections and recording of wash-out curves were made according to Forssman (3) and Jansson (6)

Curves obtained after intra arterial injections were analyzed according to eqs 5 6 and 7 When using the Zierler method for curve analysis the area under the linearly written wash out curve was determined using a

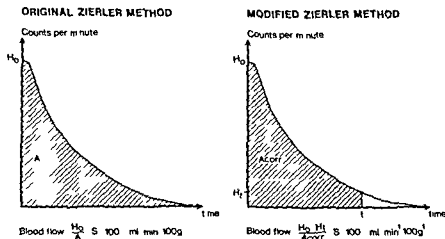



*Fig 1* The linearly written wash out curve after a brief injection of isotope analyzed according to the original Zierler method left and to the modified Zierler method right

planimeter taking the mean of five measurements In calculations according to eq 6 the area of the tail of the curve was extrapolated according to Zierler s own sugges tion The tail area was calculated by inserting into eq 6 the value of the height of the curve minus background at the end of the recording as a rule 20 min and the blood flow value for the tail portion of the curve calculated from the semilog curve and solving the equation for area

In calculations according to eq 7 all activity after 0 min was disregarded

Percentage weights of compartments were calculated according to eq 4 From 75 experiments 19 curves were obtained that were of a technical quality to allow analysis by all three methods

Curves after local injections were analyzed according to the initial slope method and to eq 2 If the curves had a very steep fall in the beginning they were replotted with the time scale enlarged up to eight times the conventional (from 1 min=+ cm to 1 min=16 cm) in order to make a detailed analysis of the very first portion possible

Local injections via the serosa were made during laparotomy in myoma cases Local injections via the mu cosa were made in two groups of volunteers one group comprising 5 castrated women under hormone treatment one comprising 6 menstruating young women Fifty re cordings that allowed both types of curve analysis were included in this part of the study

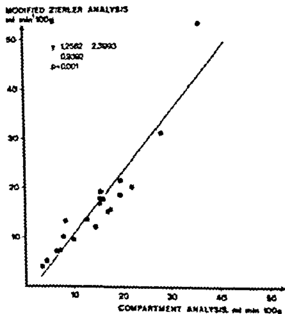



*Fig 3* Comparison between total blood flow according to compartment analysis (abscissa) and modified Zierler analysis (ordinate)

## RESULTS

*Intra arterial injections* The equation for the re gression line describing the relation between com partment analysis (eq 5) and Zierler analysis (eq 6) is $y=0\ 9772x-0\ 5921$ correlation coefficient 0 9863 ($p<0\ 001$) The individual values are plotted in Fig 2 The regression line describing the relation be tween compartment analysis and modified Zierler analysis (eq 7) is $y=1\ 2582x-2\ 3993$ correlation coefficient 0 9392 ($p<0\ 001$) (Fig 3)

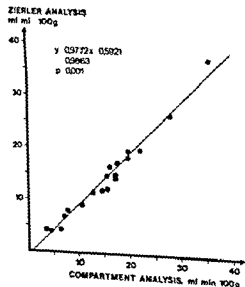



*Fig 2* Comparison between total blood flow according to compartment analysis (abscissa) and original Zierler method (ordinate)

The mean percentage weights of compartments were in 7 cases with three-compartment curves very quick compartment 5 0 range 1 3 to 8 9% quick compartment 21 3 range 10 4 to 37 3% and slow compartment 73 7 range 57 9 to 84 3% The mean values for blood flow in the compartments in the same cases were very quick compartment 198 9 range 97 to 400 quick compartment 32 7 range 24 2 to 44 1 and slow compartment 5 1 range 2 4 to 8 7 all values in ml $min^{-1}$ 100 $g^{-1}$

In 14 cases with two-compartment curves the corresponding values were percentage weight of quick compartment 21 8 range 1 2 to 49 3% blood flow quick compartment 31 9 range 13 9 to 44 1 and slow compartment 6 1 range 1 7 to 10 1 ml $min^{-1}$ 100 $g^{-1}$

*Local injections* The equation for the regression line for initial slope analysis as a function of com partment analysis (eq 2) is $y=0\ 8679x-1\ 3412$ cor relation coefficient 0 9665 ($p<0\ 001$) (Fig 4) The mean percentage weights of compartments were

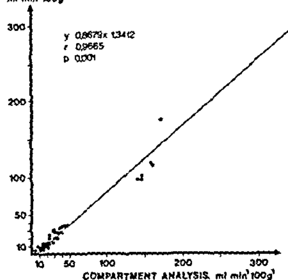



*Fig 4* Comparison between total blood flow according to compartment analysis (abscissa) and initial slope method (ordinate)

*17 cases with three-compartment curves* very quick compartment 61 9 range 32 1 to 90% quick compartment 33 8 range 9 2 to 65 0% and slow compartment 4 3 range 2 7 to 82 8% The mean compartment blood flows were in the same cases *very quick component 259 2* range 179 7 to 373 6 quick compartment 47 9 range 32 3 to 80 9 and compartment 3 6 range 1 6 to 8 1 all values in min⁻¹ 100 g⁻¹

In 23 two-compartment curves the corresponding values were *percentage weight quick compartment* 59 0 range 17 2 to 97 3% blood flow quick compartment 38 0 range 19 4 to 64 7 slow compartment 7 0 range 0 6 to 17 3 ml min⁻¹ 100 g⁻¹

## DISCUSSION

The application of the isotope clearance method for measuring uterine blood flow presents some special problems of both theoretical and technical nature The wash-out curves after both local and intra arterial injections of tracer usually are of the multi exponential type It has been possible to identify three different curve components However compartment analysis requires the hypothesis that the human non pregnant uterus is a three compartment organ In order to avoid the possibly false assumption of a certain number of compartments other methods for curve analysis were used in this study

Curves after intra arterial injections were analyzed according to Zierler and curves after local injections according to the initial slope method

The Zierler method requires exact recording of the maximum height of the curve after a short injection The curve then preferably should be followed down to background level Intra arterial injections were made during a very short period of time and as a rule the top value was recorded with sufficient accuracy For practical reasons however it has not been possible to follow the curve down to background level The extrapolation technique used in *this study for determining the tail area is in agreement* with Zierler's suggestion and should not introduce *too large an error*

Since often rather large amounts of isotope remain in the uterus at the end of the registration *period the modified technique using only the first* twenty minutes of elimination seems to lead to erroneously high values In a theoretical analysis of the degree of overestimation of cerebral blood flow by the modified Zierler analysis Olesen et al (9) found values that are similar to those of the present study

The good correlation between values calculated according to the original Zierler method and values from the compartment analysis leads to the conclusion *that although it has not been shown that values* for individual compartments have any distinct physiological meaning they may be used for calculation of total uterine blood flow

Local injections are made during a longer period of time than intra arterial injections The syringe containing isotope together with the injection needle and its isotope content are often seen by the detector disturbing the very first part of the recording These two factors prevent use of the Zierler *method after local injections*

The initial slope of the wash-out curve has been shown to be a measure of mean blood flow in a composed tissue under circumstances of equal isotope saturation of all compartments (5) No as*sumptions as to number or magnitude of compart*ments have to be made However for technical reasons it may often be difficult to identify the relevant part of the wash-out curve The present observation of good correlation between compartment analysis values and initial slope values therefore indicates that curves might be analyzed by the

compartment method if there are problems in drawing the initial slope with sufficient exactness

An interesting problem is that of differences in the figures for individual compartments obtained with the different methods for isotope injection With both local and intra arterial injections three curve components have been seen Their perfusion magnitudes are similar irrespective of type of injection Only a few measurements have been made using both local and intra arterial injection technique in the same uterus In these cases however good agreement between values for perfusion of compartments is seen The relative weights of compartments however are markedly different giving differences of total blood flow values If the hypothesis of the uterus being composed of three compartments with different perfusion is true then the labelling of these compartments must be very different with the two methods of isotope administration In the intra arterial injections the slow compartment dominates In local injections two patterns are seen In one seen only after injections via the mucosa there is a very quick component comprising over 60% of the weight of the labelled region In the other pattern seen both after injection via the mucosa and the serosa only two compartments may be identified Here the moderately quick component dominates comprising about 60% of the labelled tissue weight

If we still adhere to the three-compartment model different explanations for these differences are possible In intra arterial injections it may be that the slow compartment is over represented because of for instance labelling of perivascular fat with a very high capacity for binding Xenon

Local injections may preferentially label well perfused regions Connective tissue for instance may offer greater resistance to the spread of the injection than smooth muscle and endometrium The observation that no cases with an initial very quick component are seen after injections via the serosa is interesting and needs further study It may indicate that the very quick compartment is located very near the uterine cavity and is not so easily reached by isotope when the injection is made via the serosa as when it is made via the mucosa

The conclusions to be drawn from this study could be summarised as follows Multiexponential wash-out curves after both intra arterial and local injections into the human non pregnant uterus may be analyzed without making assumptions of a certain number of in parallel compartments The methods used then are the stochastic analysis according to Zierler and the initial slope method If it is assumed that the uterus is a three-compartment organ it is also possible to calculate mean blood flow within the isotope labelled region by using relative compartment weight and compartment blood flow Good agreement is seen between values calculated according to the two principles

Relative weights of the individual compartments however are markedly different within regions labelled by intra arterial injections and those labelled by local injections Compartment blood flows on the contrary are in the same order of magnitude with both types of labelling Thus the three compartment model seems to be useful in determining mean blood flow The value of individual compartment blood flows and relative compartment weights remains to be demonstrated

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*Submitted for publication Sept 22 1974*

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Acta Obstet Gynecol Scand 54 485–489 1975

# INTRA URETERIC PRESSURES IN WOMEN UNDERGOING HYSTERECTOMY FOR CARCINOMA OF THE UTERINE CERVIX

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*Abstract* Ureteric function in five women undergoing Wertheim hysterectomy for carcinoma of the uterine cervix was assessed from intra ureteric pressure recordings made with a new technique No notable difference was found in frequency rate or amplitude between the uretero-peristaltic waves recorded immediately before and after dissection of the ureter In three cases however retro-peristalsis occurred and in two of them it persisted throughout the operation The presence of retrograde peristalsis may indicate that the peristaltic activity of these ureters was disturbed by the operative manipulation Ureteric stasis was also demonstrable by radiographic techniques in those cases in which retro-peristalsis occurred during the operation but not in those in which it was absent

Ureteric stasis is not an uncommon complication of Wertheim hysterectomy for carcinoma of the uterine cervix (14) It is difficult to predict the later course of such early postoperative urinary stasis it may regress spontaneously or progress and lead to hydronephrosis with reduction of the renal parenchyma and impaired renal function Prediction of the further course requires a fairly close follow up of the urinary flow with urography and/or isotope renography (6 7 9 10 11 13 14)

Early postoperative ureteric stasis after Wertheim hysterectomy has been ascribed to postoperative oedema which is accentuated by the remova of the lymph nodes (7) or to the dissection and consequent denervation of the lower segment of the ureter and urinary bladder (2 4 5 9) A combination of both possibilities and supervening infection are also plausible explanations (4 7 10)

When ureteric stasis occurs at a later stage (>6–12 months) it is probably due to fibrous strictures or to recurrence of the tumour To test whether the above operation leads to functional disturbances of the lower segment of the ureter it is necessary to examine ureteric function immediately at the end of the operation before any oedema and infection has had time to develop

In the present investigation ureteric function therefore was examined immediately before during and after a Wertheim hysterectomy in five patients suffering from carcinoma of the uterine cervix

## CLINICAL MATERIAL AND METHODS

The material consisted of five women with carcinoma of the uterine cervix stage Ia–Ib The pre and postoperative findings as well as the intraureteric pressure recordings are summarized in Tables I and II

The pressure recording technique used has been described in detail elsewhere (15) and is therefore only briefly outlined here With a specially designed multi channel catheter connected to strain gauge transducers (Fig 1) the intra-ureteric pressure was recorded simultaneously at 3 levels 10 cm apart Thanks to the design of the catheter it was possible to assess ureteric peristals s within and above the operation site

With the patient under anaesthesia the catheter was introduced into the ureter with the aid of Wolff uretero-cystoscope The lowest pressure sensing section (A 3) was situated 4 cm from the ureteric orifice in four cases and at 2 cm from orifice in one The cystoscope was then withdrawn and the urinary bladder was drained through a Foley catheter connected to a collecting bag At the external urethral orifice the ureteric catheter was fastened to the Foley catheter

In three cases the ureteric catheter was introduced on the right side and in two on the left The pressures were recorded for the last 15 minutes before and for the first 15–30 minutes after the dissection of the ureter (In three cases the pressures were recorded also during the dissection of the ureter )

Table I *The age and diagnosis of the five patients The results of the pre and postoperative renal function investigations (intravenous pyelography radioisotope renography creatinine/blood and urea) The table also shows whether radiotheraphy has been given or not Note that in patients 3 4 and 5 where retro-peristalsis was noted during the operation ureteric stasis was demonstrated in the urogram and renogram one week postoperatively*

| Patient no | Diagnosis | Age | Ra | Urography | Renography | Cr/s | Urea | |
|---|---|---|---|---|---|---|---|---|
| 1 | ca colli uteri I a | 57 | – | Normal | Normal | 1 0 | 28 | Preop |
| | | | | Normal | Normal | 1 1 | 30 | Postop |
| 2 | ca colli uteri I b | 36 | Brachy radium×3 | Normal | Normal | 1 1 | 29 | Preop |
| | | | | Normal | Normal | 0 9 | 27 | Postop |
| 3 | | 62 | Brachy radium×2 | Normal | Normal | 1 2 | 30 | Preop |
| | | | | Obstruction dx | Obstruction dx | 1 1 | 29 | Postop |
| 4 | ca colli et corporis uteri | 51 | Brachy radium×3 | Normal | Normal | 0 9 | 32 | Preop |
| | | | | Obstruction sin | Obstruction sin | 0 9 | 43 | Postop |
| | | | | | Obstruction bilat | 0 9 | 43 | Postop |
| 5 | ca colli uteri I b | 44 | Brachy radium×3 | Normal | Normal | 1 0 | 28 | Preop |
| | | | $^{60}Co$ 4 500 r | Obstruction bilat | Obstruction bilat | 1 1 | 30 | Postop |

Various factors are capable of interfering with the operative recordings Efforts were therefore made to maintain as steady a state as possible from the first to the second recording No drugs were given during the dissection of the ureter and as the dissection was not complicated by any notable loss of blood no blood or extra fluid was given After the pressures had been recorded the urinary bladder was filled and via the cystoscope urine was seen to flow along the catheter into the urinary bladder The ureteric catheter was afterwards withdrawn and calibrated in the same way as before the examination (15)

Surgical technique During the operation the ureter was [illegible] from the external iliac vessels down to its en into the urinary bladder It was thus exposed along about 8 cm of its length The uterine artery was ligated lateral to the ureter and the parametria and the cardinal ligaments were divided as far laterally as possible After the urinary bladder had been pushed downwards the uterus was exsturpated with a 3–4 cm vaginal cuff As previously described the ureteric pressures were recorded immediately before (during three cases) and just after the dissection of the ureter in question

## RESULTS

No certain numerical difference was found between the preoperative and the postoperative uretero-

Table II *Numerical data of the ureterometry*

| Patient no | Ureter examined | Peristaltic travel rate (mm/sec) | Contractions per minute | Direction of peristalsis | Peristaltic pressure amplitude in cm $H_2O$ at distances from bladder indicated (cm) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 2 | 4 | 12 | 14 | 22 | 24 | |
| 1 | dx | 40 | 7 | Antegrade | 15 | | 20 | | 25 | | Before op |
| | | 40 | 5 | Antegrade | 12 | | 25 | | 30 | | After op |
| 2 | dx | 30 | 7 | Antegrade | | 40 | | 22 | | 26 | Before op |
| | | 30 | 9 | Antegrade | | 35 | | 30 | | 26 | After op |
| 3 | dx | 40 | 7 | Antegrade | | 25 | | 28 | | 20 | Before op |
| | | 33 | 7 | Retrograde | | 35 | | 25 | | 25 | After op |
| 4 | sin | 33 | 5 | Antegrade | | 35 | | – | | 22 | Before op |
| | | 42 | 5 | Retrograde | | 45 | | – | | 25 | After op |
| 5 | sin | 35 | 8 | Antegrade | | 20 | | 25 | | – | Before op |
| | | 38 | 7 | Retrograde | | 25 | | 25 | | – | After op |

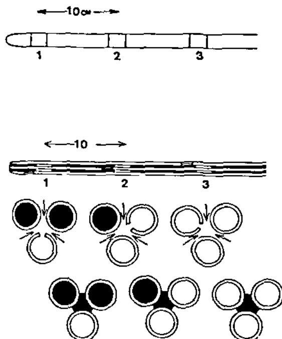



*Fig 1* Catheter used for transmission of the intra ureteric pressures Pressure is transmitted from three points 1 ? and 3 which are 10 cm apart The intra-ureteric pressure amplitude recorded at these three levels are also indicated A 1 A 2 and A 3

metry As can be seen from Table II the frequency the rate and the amplitude of the peristaltic waves were about the same after as before the operation Neither was any difference demonstrable between the left and the right side (Table II) Fig 2 a and b demonstrate pressure diagrams from a patient just before and after the operation As can be seen the peristaltic waves moves from the renal pelvis towards the urinary bladder i e there is a normal *antegrade peristalsis In three patients however* the normal antegrade peristalsis was changed during the operation to an abnormal retrograde peristalsis This is demonstrated in fig 3 *a* and *b* The retro-peristalsis persisted in two of those three patients throughout the measuring period (>30 min)

In those cases where retro-peristalsis occurred a postoperative renography or urography demonstrated the presence of an outflow obstruction on the actual side (Table I)

## DISCUSSION

The pressure recording section A 3 (the lowermost recording level) was in four cases situated 4 cm from the ureteric orifice and in the fifth case 2 cm from the orifice The dissected segment of the ureter as measured during the operation was about 8 cm The lowermost pressure recording section thus clearly lay within the dissected segment of the ureter As can be seen from Table II and the diagrams in Figs 2 and 3 very little postop change in amplitude frequency or rate of the peristaltic waves was noted in this part of the ureter These data do not favour the idea of a denervation of the ureter as a result of a Wertheim hysterectomy

In the absence of notable differences between the pre and postoperative uroterometry one might imagine that the operation had no appreciable effect on the ureteric peristaltic activity It is however remarkable that in three of five cases retro-peristalsis was observed after the dissection of the

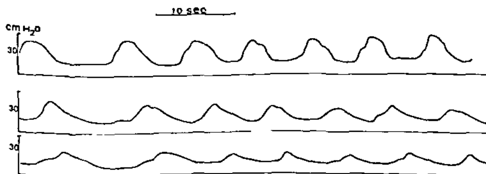



*Fig 2 a* Normal antegrade peristalsis before dissection of the ureter The top curve corresponds to the pressure recording sensing section A 1 situated 2 cm from the urinary bladder Middle curve is recording the intra ureteric pressure 10 cm downwards (12 cm from the urinary bladder) and bottom curve is recording the pressure 2 cm from the urinary bladder

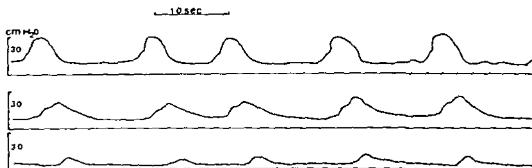



*Fig 2b* The same patient immediately after the dissection of the ureter Still there are normal antegrade peristaltic waves The same recording levels as in Fig 2a

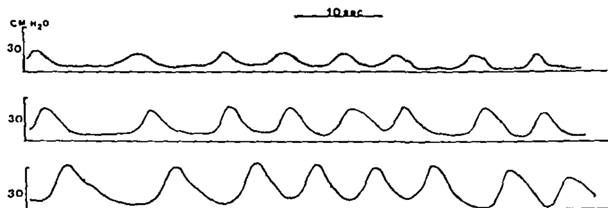



*Fig 3a* Normal antegrade peristalsis from a patient immediately before the dissection of the ureter The top curve is recording the pressure 24 cm from the urinary bladder middle curve corresponds to intra ureteric pressure 10 cm downwards (14 cm from the urinary bladder) and the lowermost curve is recording the pressure 4 cm from the urinary bladder

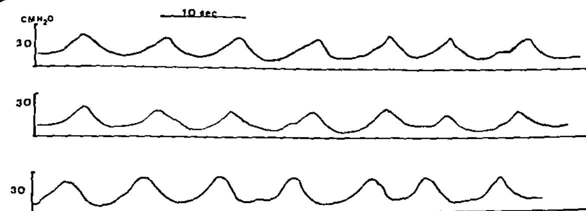



*Fig 3b* The same patient and the same recording levels as in Fig 3a but after the dissection of the ureter Note that retro-peristalsis has occurred during the dissection of the ureter The retro peristalsis persisted during the whole recording period (>30 min )

ureter and that this retro peristalsis was still demonstrable at the end of the recording period (>30 min) In these three cases where the retro peristalsis occurred in association with dissection of the ureter normal antegrade peristalsis had been observed before the dissection Irritation by the catheter can therefore hardly have been the main cause of the retro peristalsis Otherwise retro peristalsis would presumably have occurred in association with insertion of the catheter

Reports of retro-peristalsis are very scanty Enhorning and Weaver (3) have described the development of retro-peristalsis during manipulation of the lower segment of the ureter in dogs undergoing laparotomy Similar observations have also been reported by Kiil (8) Mayer et al (11) who used cine urography observed retro-peristalsis in one case shortly after a Wertheim hysterectomy

It has been demonstrated elsewhere that long lasting retrograde peristalsis can result in a functional obstruction of the flow of urine through the ureter (15)

From this investigation it can not be determined how long the retro peristalsis persisted or to what extent retro-peristalsis may contribute to early transient postoperative ureteric obstructions of the urinary flow after a Wertheim hysterectomy An answer to this would require repeated pressure recordings during the early postoperative course with considerable ri ks of urinary infection However isotope renography and urography 1 week after the operation demonstrated ureteric obstruction in those patients where retro-peristalsis had occurred during the operation (Table I) In all the patients the ureterometry the renogram and/or urogram became normal within 4 months after the operation

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*Submitted for publication Dec 3 1974*

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# INFORMATION RECEIVED BY F I G O ON THE FOLLOWING CONGRESSES

*VI Panama Meeting of Obstetrics and Gynecology Panama Republic of Panama January 15–20 1976*

Themes relating to Obstetrics and Gynecology For information contact the Sociedad Panamena de Obstetricia y Ginecologia Apartado 1434 Panama Zona 1

*VII New Zealand Congress of OB/GYN Nelson New Zealand February 17–21 1976*

Themes Medico-social and economic aspects of antenatal care antenatal diagnosis of fetal abnormality and the at risk fetus planned deliv ry and control of labor premature labor intrapartum assessment and management changes in the management of gynaecological malignancies For information contact Dr G R Duncan Clinical School Wellington Hospital Wellington

*XVI General Assembly of the Association of Obstetrics and Gynecology of the Republic of China Taipei Taiwan Republic of China February 1976*

Themes relating to Obstetrics and Gynecology For information contact Professor Yao-Wen Wang Department of Obstetrics and Gynecology National Taiwan University Hospital Taipei Taiwan 100

*VI Congress of the German Democratic Republic Society of Obstetrics and Gynecology Karl Marx Stadt German Democratic Republic May 17–21 1976*

Themes Infection in Obstetrics and Gynecology medical and social problems relating to human reproduction endocrinology in reproduction plastic and reconstructive operations in gynecology prematurity and dysmaturity For information contact the Secretariat of the Congress Frauenklinik des Bezirkskrankenhauses Flemmingstr 4 GDR—909 Karl Marx Stadt

*ymposium CSSR–NDR Gynecological and cal Societies Frantiskovy La ne oslovakia May 23–24 1976*

mes relating to Obstetrics and Gynecology For information contact Doc MUDr Karel Vesely DrSc Londynska 41 120 00 Praha 2

*XXVIII Annual Meeting of Japan Society of Obstetrics and Gynecology Matsumoto City Nagano Pref Japan May 23–26 1976*

Themes relating to Obstetrics and Gynecology For information contact Professor Shoji Iwai Shinshu University School of Medicine Matsumoto Nagano

*XIV Meeting of the Asociacion Ginecologica Espanola Malaga Torremolinos Spain May 1976*

Themes Pathology of the breast from the gynecologist s point of view physiopathology of the amniotic fluid infection in obstetrics For information contact Professor Dr Julio Cruz y Hermida Secretary Asociacion Ginecologica Espanola Villanueva 11 2° Madrid 1

*III Indonesian Congress of Obstetrics and Gynecology Medan North Sumatra Indonesia June 7–11 1976*

Themes Latest progress in family planning (fertility) social obstetrics obstetrics education for para medis obstetrics pathology gynecology cancer Free Communications For information contact the Secretariat of the Congress P O Box 576 Medan North Sumatra

*Meeting of the Society of Obstetricians and Gynaecologists of Canada Toronto Ontario Canada June 8–12 1976*

Themes relating to Obstetrics and Gynecology For information contact the Secretary Society of Obstetricians and Gynaecologists of Canada 367 Lytton Blvd Toronto Ontario M5N 1R9

*Congress of the South African Society of Obstetricians and Gynaecologists Durban South Africa September 13–16 1976*

Themes relating to Obstetrics and Gynecology For information contact the Hon Secretary South African Society of Obstetricians and Gynaecologists Faculty of Medicine P O Box 667 Pretoria 0001

*Meeting of the German Federal Republic Society of Obstetrics and Gynecology Hamburg German Federal Republic September 28 – October 2 1976*

Themes Breast perinatology (amniotic fluid diagnosis) contraception conception—threatened abortion psycho-social problems—climacterium endocrine disorders in teenagers complications during laparoscopy and culdoscopy For information contact Professor Maass Univ Frauenklinik 2 Hamburg Eppendorf

*VIII Congress of Gynecologists and Obstetricians of Yugoslavia Portoro Yugoslavia October 7–9 1976*

Themes Monitoring of the fetus during pregnancy and delivery physiology and pathology of residual ovarium nephro-urologic complications in gynecology Round Table discussions Diabetes mellitus in pregnancy the role of the gynecologist in breast cancer detection postgraduate education in gynecology and obstetrics Fo information contact Dr Meta Hren II Secretary of th Association of Gynecologists and Obstetricians o Yugoslavia Slajmerjeva 3 Ljubljana.

*VIII World Congress of Obstetrics and Gynecology Mexico City Mexico October 17–23 1976*

Themes Environment and reproduction benign disease of the female breast infection in gynecology an obstetrics advances in perinatology congenital mal formations and acquired defects of the Mullerian duct and of the vulva Free communications For informatio contact Dr C MacGregor Secretary General of th Congress Av Baja California 317 Mexico 11 D F

Acta Obstet Gynecol Scand 54 491–496 1975

CASE REPORTS

# INTRAVASCULAR COAGULATION IN PREGNANCY— TREATMENT WITH HEPARIN

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Lennart Jacobson Lars Holmberg and Sten Ohrlander

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*Abstract* Two pregnant women with a history of miscarriages or premature labour are described In the 25–27th week of pregnancy routine examinations showed high levels of FDP in serum Subsequent extensive coagulation studies revealed a positive ethanol gelation test low levels of fibrinogen plasminogen $\alpha_2$-macroglobulin and P&P and high level of AHF related protein for the stage of pregnancy i e findings indicating abnormal proteolysis with activation of the coagulation and fibrinolytic system Continuous intravenous heparin treatment caused the coagulation system to return to normal throughout the rest of pregnancy Both women gave birth to healthy babies without any complications But the placenta showed numerous infarcts The heparin treatment may have prevented later development of placental dysfunction

Disseminated intravascular coagulation has been reported to occur in pregnant women with preeclamptic toxaemia and eclampsia (3 15 23 25 26) Fibrin deposits in the kidney liver and lungs have been demonstrated in these conditions (16 25 27) as well as increased fibrinogen catabolism (27) Fibrin deposits have been shown to occur in the kidney also in other complications of pregnancy such as abruptio placentae (25)

This paper describes two women with previous miscarriages and premature labour of unknown cause They were now pregnant again Coagulation studies in the third trimester revealed changes suggesting disseminated intravascular coagulation Treatment with heparin caused the coagulation status to return to normal and both women gave birth to healthy babies at term without any complications

## METHODS

*Platelet and coagulation studies* Platelet count Duke bleeding time coagulation time activated partial thromboplastin time (APTT) one stage prothrombin time prothrombin + factor VII + factor X (Owren s P&P test) factor VIII (AHF) (biologic activity) and fibrinogen were determined according to methods described earlier (19 22) *Factor VIII* was immunologically determined according to Holmberg & Nilsson (12) *Antithrombin III* was determined immunochemically by the rocket method of Laurell (9) The *ethanol gelation test* was used for determining fibrin monomers according to Godal et al (7)

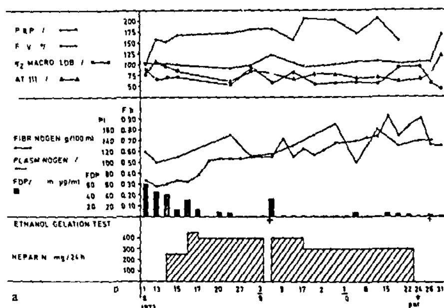
*Fibrinolytic studies* The *fibrinolytic activity* of plasma and resuspended euglobulin precipitate was measured on unheated bovine fibrin plates as described by Nilsson & Olow (21) *Plasminogen* was measured by an immunochemical method (4) *Fibrin/fibrinogen degradation products* (FDP) were determined with the immunochemical method of Niléhn (8 18) in serum samples obtained from blood collected with EACA and thrombin *Inhibitors of plasminogen activation* (urokinase inhibitors) and *$\alpha_2$-macroglobulin* ($\alpha$ M) were determined as previously described (10)

## CASE REPORTS

*Case I* (A G ) The patient was a 33-year-old woman with a history of three miscarriages in the 2nd to 4th month of pregnancy Histological examination had not revealed any changes capable of explaining the abortions Chromosome analysis revealed normal karyotypes in both the patient and her husband Hysterosalpingography showed nothing remarkable

After a brief period of depression she became pregnant again in 1973 (last menstruation on 7th February) Some months before pregnancy she had a urinary infection and long term prophylactic therapy was started with sulphonamides which were replaced by ampicillin during the last few months of pregnancy With the exception of some episodes of slight vaginal bleeding and a transient tendency to uterine contractions in the first trimester the first half of her pregnancy was uneventful

Because of her previous abortions she was admitted to the department of obstetrics for examination in the 25th week of pregnancy She felt well and showed no signs of toxaemia, no oedema albuminuria or increase in blood



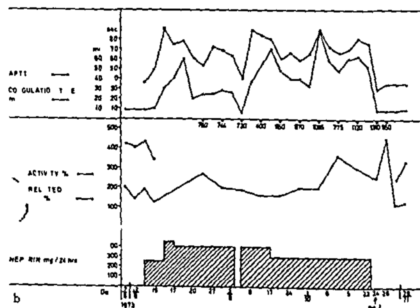
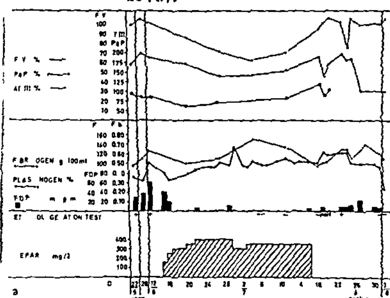


*Fig 1 a b* The course in case 1

pressure Urinary oestriol and human placenta lactogen (HPL) were normal Also SGOT and SGPT and serum bilirubin were normal The only pathological finding was an increased concentration of FDP in the serum 48 µg/ml In the following week FDP remained increased between 45–67 µg/ml The coagulation system and the components of the fibrinolytic system were therefore examined (26th week) The concentration of fibrinogen proved low and the ethanol gelation test was positive indicating activation of the coagulation system

Anticoagulant therapy with heparin was therefore started in the 27th week of gestation Heparin was given by continuous intravenous drip monitored by an electronic drip counter The doses were adjusted to keep the APTT at about 70–100 seconds (normal range 35–45 seconds) The woman usually received 15 mg (1 500 units) heparin/hour (see Fig 1 *a b*)

In the 30th week of pregnancy heparin was withdrawn for 2 days but afterwards continued to the 38th week No clinical signs of toxaemia appeared Urinary oestriol *HPL*



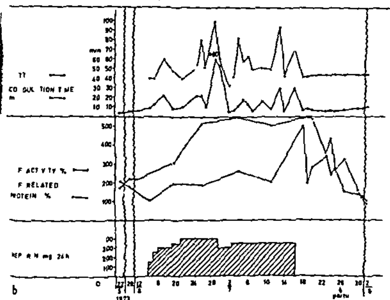




*Fig. 2 a, b.* The course in case 2.

and fetal biparietal diameter were normal. 48 hours after the withdrawal of heparin (23rd Oct) labour was induced by artificial rupture of the membranes and administration of oxytocin intravenously. Normal delivery occurred after 8 hours normal labour. The child was normal; it weighed 3 160 g and had an Apgar score of 9. The placenta weighed 600 g and its surface showed gross scattered white infarcts the size of hazelnuts. Histological examination revealed abundant fibrin deposits, mainly in the fetal part of the placenta.

*Case 2* (M. O.). The patient was a 22 year-old woman who had had a premature delivery in 1971. The child weighed 1 300 g at birth. It died after 3 weeks. Autopsy revealed nothing remarkable, and the diagnosis was immaturity. Unfortunately the placenta was not examined histologically.

In 1972 the patient was pregnant again. In the first 8 weeks she had some episodes of slight vaginal bleeding which ceased spontaneously. It was then noted that the uterine cervix was already one finger wide. To prevent the premature onset of labour the patient was admitted to the obstetrical department in the 26th week of pregnancy. On admission the patient was apparently healthy; she had no signs of toxaemia. Analyses of electrolyte, serum

creatinine SGOT SGPT serum bilirubin urinary oestriol and HPL showed nothing remarkable There was no anaemia However routine examination of serum FDP revealed values of 20–28 μg/ml This finding prompted extensive analysis of the coagulation factors and the components of the fibrinolytic system The fibrinogen level was low and the ethanol gelation test was positive Treatment with heparin was therefore initiated (31th week of pregnancy) It was administered intravenously by means of an electronic drip counter The doses were adjusted to keep the APTT between 70–100 seconds The patient usually required 15 mg (1 500 units) heparin/hour (see Fig 2 *a* *b*) The last 6 days the heparin was given subcutaneously No signs of toxaemia developed The urinary oestriol remained within normal limits throughout gestation

Heparin was withdrawn in the 37th week of gestation and 6 days afterwards labour was induced by rupturing of the membranes Delivery was normal the child had an Apgar score of 9 birthweight 2 700 g The placenta weighed 470 g and its surface showed infarcts

Six days after delivery the mother and child left hospital without any complications

## RESULTS OF THE COAGULATION ANALYSES

*Case I* (A G) (Fig 1 *a* *b*) At routine examination in the 27th week of pregnancy the amounts of FDP in serum had increased to 67 μg/ml Fibrin monomers were demonstrated in the circulating blood (positive ethanol gelation test) The fibrinogen level was low namely 0 28 g/100 ml The levels of P&P (100%) $\alpha_2$M (65%) and plasminogen (100%) were low for pregnancy The platelet count factor V and inhibitors of the plasminogen activation were normal No increased fibrinolytic activity as assayed on The levels of AHF measured biologi ly were normal for this stage of pregnancy 00%) But the AHF related protein was increased (430%) The antithrombin III level was normal

Heparin therapy was started in the 27th week of pregnancy 2 days later the ethanol gelation test became negative and within the following 10 days the serum FDP disappeared Heparin treatment was discontinued in the 30th week The following day the patient again had FDP and the ethanol gelation test was positive Heparin treatment was resumed and the FDP again disappeared and the ethanol gelation test became negative During the treatment fibrinogen successively rose to 0 92 g/100 ml The plasminogen increased to 165% The P&P levels rose to 180% AHF related protein rose to reach very high values (>1 310%)

*Case 2* (Fig 2 *a* *b*) Repeated routine determination of FDP in the serum showed FDP in amounts increasing from 20 μg to 60 μg/ml The ethanc gelation test was positive With respect to the preg nancy (27th week) fibrinogen (0 36 g/100 ml) an plasminogen (95%) were low The platelet coun P&P and factor V were normal The AHF activit was 205% which is within normal limits for the thir trimester but AHF related protein was increased No increased fibrinolytic activity The level of ant thrombin III was normal The values found for th inhibitors of plasminogen activation were normal fo pregnancy Heparin treatment (i v ) was started i the 31st week of pregnancy

During the heparin treatment fibrinogen an plasminogen increased successively Fibrinog reached a value of 0 67 g/100 ml which is normal fo the third trimester of pregnancy After 3 days treat ment the ethanol gelation test became negative an the FDP disappeared

The AHF activity rose slightly and the level o AHF related protein was very high (>580% throughout the course of treatment This patien received heparin subcutaneously in the last weel before the delivery During that week the ethano gelation test was occasionally positive and the da after the heparin had been withdrawn it was positive During the same period the FDP reappeared in the serum

## DISCUSSION

Coagulation disorders secondary to disseminatec intravascular coagulation have been described i toxaemia (3 23 24 27) in the dead fetus syndrom (24) and abruptio placentae (25 27) In the 2 patient described in this paper coagulation disturbance were found as early as the 27th week of pregnancy The presence of fibrin monomers in the circulation the low fibrinogen and plasminogen values for th stage of pregnancy and the occurrence of FDP in th serum in high concentrations indicated a complica tion of the pregnancy leading to activation of th coagulation and fibrinolytic system i e an enhancec proteolysis with excessive or pathological formatio of fibrinogen derivatives During normal pregnanc the levels of fibrinogen plasminogen P&P (20) anc $\alpha_2$M (6) are high in the third trimester $\alpha_2$M is know to form complexes with plasmin trypsin and wit other proteolytic enzymes (6 17) The low level o $\alpha_2$M in case 1 suggests that the abnormal proteolysi in this patient was more marked than in case 2 Th finding of normal antithrombin III value on admis

sion of the patients was noteworthy Antithrombin III is known to bind thrombin and plasmin (1) and has been claimed to be decreased in disseminated intravascular coagulation (14) In our study the antithrombin III was determined immunochemically and some of the antithrombin III determined with this method may therefore be present as a complex Moreover during pregnancy synthesis may be increased The decrease in plasminogen is most probably due to activation of the fibrinolytic system as a consequence of the fibrin deposited Such a possibility has also been proposed by Davidson & Phillips (3) Activation of the fibrinolytic system also results in the formation of FDP which occurred in rather high concentrations in serum from these patients In fact it was these high concentrations that drew our attention to the possibility of a complication of the pregnancy The importance of routine determinations of FDP in serum during the last trimester of pregnancy has been stressed earlier (11) Toxaemia and hepatosis are common in the women with FDP in serum (7 11)

The presence of intravascular coagulation in these cases was corroborated further by the effect of heparin treatment This treatment resulted in almost a complete return to normal in the coagulation system The ethanol gelation test became negative and the FDP disappeared The fibrinogen plasminogen and P&P levels rose to normal levels In case 1 heparin was withdrawn after 2 weeks treatment and then the values immediately became abnormal but were again made normal by giving heparin After reduction of heparin doses and later withdrawal of the drug before labour the abnormal values promptly reappeared in case 2

The AHF values are of special interest It is known that AHF is considerably increased in many kinds of physical stress and particularly in conditions with marked tissue destruction and repair such as liver necrosis burns and metastasing cancer (13) In these situation quantitative immunological methods often give substantially higher values for the AHF related protein than a biological activity assay This is perhaps due to differences in the degree of aggregation of the protein subunits In the 2 patients and especially in case 1 AHF related protein rose to unexpectedly high levels Such an increase is probably a sign of abnormal tissue destruction Heparin treatment resulted in a complete return to normal in the coagulation system except for the AHF related protein We can thus infer that heparin did not eliminate the basic cause of the tissue destruction but prevented its effect on blood coagulation

A marked increase in infarction of the placenta has been demonstrated in toxaemia although the perivillous fibrin deposits were equally common as in normal (5 28) The placentae of the 2 patients described also showed increased amounts of white infarcts indicating impairment of the maternal decidual arterioles (28) In spite of the coagulation disturbances and the increased amounts of white placental infarcts these patients showed no clinical signs of toxaemia such as hypertension oedema or proteinuria Impaired placental function and coagulation disturbances indicating activation of the coagulation system have however also been described in other obstetrical complications probably associated with placental dysfunction for example the dead baby syndrome and abruptio placentae (24 25 27)

As pointed out above the good effect of heparin treatment which was performed without any complications suggested that the coagulation system really had been activated in these patients Although these patients showed no signs of impaired placental function on admission such a complication might have occurred if they had been left untreated One of these patients (case 1) had had previous miscarriages and the other a premature delivery of unknown cause It is possible that impairment of the placental function played a contributory role

Judging from the observations in the 2 patients described early diagnosis and heparin treatment of an activated coagulation system during pregnancy is important This may prevent development of placental dysfunction and the otherwise gloomy prognosis of such pregnancies

## ACKNOWLEDGEMENT

This investigation was supported by grants from the Swedish Medical Research Council (B74-19X 87 10 B74-19X-678-09) and Riksbankens Jubileumsfond

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*Submitted for publication May 2 1974*

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Acta Obstet Gynecol Scand 54 497–498 1975

# PREVENTION OF PREMATURE DELIVERY IN A UNICORNUATE UTERUS BY CERVICAL CERCLAGE

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*Abstract* A 26-year-old woman had two premature deliveries when 6 months pregnant Neither produced a living child A hysterosalpingogram was done confirming a diagnosis of unicornuate uterus When the patient became pregnant again a third and fourth time cervical cerclage was done in both cases Pregnancies were terminated by cesarean section because of breech presentation and malformation of the uterus and in each case a healthy living child was delivered In our view in order to prevent premature deliveries when a diagnosis of uterus malformations has been confirmed a cerclage should be performed

Due to the widespread use of the hysterosalpingography and laparoscopy congenital anomalies of the genital tract can be discovered with greater frequency than before (9) The true uterus unicornis or hemiuterus is a comparatively rare condition

An incidence of only 0 3 percent was found by Baker (1) in the cases that he reported Uterine abnormalities with the exception of infantile and hypoplastic types have an incidence of 1 420 in a series of 13 000 cases analysed by Gemmel (3) Munro-Kerr (6) stated that pregnancy is rare in the unicornuate uterus An admirable classification of developmental anomalies of the uterus has been given by Hunter (4) and our case appears to fall in the category of uterus unicornis unicorpus and unicollis Demarez (2) and Schattenberg (8) reported 32 cases upto 1940 of true unicornuate uterus

Since that time to our knowledge only 18 further cases have been reported in the literature

## CASE REPORT

P G (ref no 16 11) a 6-year-old married woman attended our Outpatient Clinic having a history of two previous mid trimester abortions when 6-months pregnant As a result she had no living children A hysterosalpingogram was made and this revealed a long narrow uterus with only one Fallopian tube on the right side which we diagnosed as a hemiuterus An intravenous pyelography was later made and showed normal kidneys

Since we know that this kind of malformation can cause premature deliveries we advised the patient that when she next became pregnant we hoped to be able to help her by doing a cervical cerclage at the beginning of the 4th month Shortly after the patient became pregnant and the cerclage was performed

In the 9th month the fetus was lying as a breech presentation and therefore it was decided to deliver her by cesarean section The section was performed in another hospital since our patient while visiting her mother there appeared to be in labour The patient was delivered of a healthy female child the first living after 3 pregnancies

After two years she conceived again and at the end of the 4th month we again did a cervical cerclage The antenatal period was uneventful and the pregnancy grew normally till the 38th week At that time she was admitted to our department with a view to repeat elective cesarean section sin e there was again a breech presentation

The examinations revealed a well-healed midline subumbilical surgical scar By palpation we found the uterus to be the size of a 38 week pregnancy with a breech presentation The fetal heart rate was normal Vaginal examination showed that the cervix was high deviated to the right and softened The internal os did not admit the tip of a finger X ray confirmed the breech presentation and showed that the lower femoral epiphyseal ossification centre has appeared which verified that the fetus was mature Consequently we considered this the right time to perform a lower segment cesarean section after prior removal of the cervical cerclage

The uterus appeared twisted to the right The operation was completed and a living female infant was delivered which weighed ? 850 g The placenta was removed but one cotyledon was adherent high on the anterior wall so we did a curettage with a macrocurette Further examination of the abdomen after closure of the uterus showed no trace of the left uterine vessels The uterus appeared to be unicornuate with one normal tube and broad round and ovarian ligaments on the right side only At the left



*Fig 1* Hysterosalpingogram showing a unicornuate uterus with a single patent right tube

nal ring we found the second part of a small fibrous uterus 4 cm long and at its left corner there was a long but very narrow left ovary 5 0×0 3 cm in length with a short tube and a well-developed fimbrial end and round ligament There was no connection to the right uterus No abnormalities of any other abdominal organ were found

Post-operative progress was good The infant showed no congenital abnormalities

## COMMENT

Only 12 cases are mentioned in the literature we were able to study in which there was a total absence of one ovary Fallopian tube and broad ligament It is remarkable that in 3 of these cases this occurred on the left side and in 9 cases on the right side In 11 other cases the second ovary was ectopic and located above the pelvic brim The ovary in general was usually longer and narrower on the abnormal side When the Fallopian tube was present it was almost always a short portion of the fimbriated end The round ligament usually existed as a small and poorly developed structure attached to the cervical portion of the uterus The broad ligament was also absent on the defective side although occasionally a small part of it could be observed The ovarian ligament was found to ex tend down toward or into the internal inguinal ring When the ovarian vessels were traced they found to arise from the aorta or to enter the cava In all cases reported external genitalia normal Menses were either normal or irregul painful

Philipp (7) also commented that the right frequently more developed than the left as case

From the X ray a so-called hemiuterus also be seen whereas in the case of a bicornis or duplex where the rudimentary does not fill up it cannot be seen

The explanation for the higher percent breech presentation is that the spindle shape uterus unicornis appears to favour this positi in both pregnancies we describe (5 10)

The new case we are here presenting is of s interest due to the fact that two living childre delivered by cesarean section after two pre deliveries without a living child before the c cerclage was performed

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*Submitted for publication May 8 1974*

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